

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification⁶ : C12N 15/54, 9/12, 15/11, 5/10, C07K 16/40, G01N 33/573, C12Q 1/48, 1/68, A61K 31/00</p>	<p>A1</p>	<p>(11) International Publication Number: WO 99/32637</p> <p>(43) International Publication Date: 1 July 1999 (01.07.99)</p>
<p>(21) International Application Number: PCT/GB98/03793</p> <p>(22) International Filing Date: 17 December 1998 (17.12.98)</p> <p>(30) Priority Data: 9726851.0 19 December 1997 (19.12.97) GB</p> <p>(71) Applicant: ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB).</p> <p>(72) Inventors: NORRIS, Tyrell, Errick; 1800 Concord Pike, P.O. Box 15437, Wilmington, DE 19850-5437 (US). MOORE, William, Craig; 1800 Concord Pike, P.O. Box 15437, Wilmington, DE 19850-5437 (US). SILBERSTEIN, David, Shay; 1800 Concord Pike, P.O. Box 15437, Wilmington, DE 19850-5437 (US).</p> <p>(74) Agent: PHILLIPS, Neil, Godfrey, Alasdair; Zeneca Pharmaceu- ticals, Intellectual Property Dept., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).</p>	<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>	
<p>(54) Title: HUMAN SIGNAL TRANSDUCTION SERINE/THREONINE KINASE</p>		
<p>(57) Abstract</p> <p>A human signal-transduction kinase polypeptide is described which is expressed at a particularly high level in tissues of the human immune system. A full length cDNA which encodes the signal transduction serine/threonine kinase polypeptide is disclosed as well as the interior structural region and the amino acid residue sequence of the native biological molecule. Methods are provided to identify compounds that modulate the biological activity of the human Ste20-like serine/threonine signal transduction kinase.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

HUMAN SIGNAL TRANSDUCTION SERINE/THREONINE KINASE

Priority is claimed under 35 USC § 119(a) from UK Application GB 9726851.0
entitled HUMAN SIGNAL TRANSDUCTION SERINE/THREONINE KINASE, filed December
5 19, 1997; the entire disclosure of which is incorporated by reference.

Field of the Invention

The present invention relates to nucleic acid and amino acid sequences pertaining to a
novel human signal transduction serine threonine protein kinase and to the use of these
sequences to identify compounds that modulate signal transduction activity of the native
10 biomolecule. The invention is also related to the diagnosis, study, prevention, and treatment
of pathophysiological disorders related to signal transduction.

Background of the Invention

Cellular response mechanisms to stress are fundamentally important to the human
immune system. Stress responses represent carefully devised cellular defense mechanisms
15 which were developed at an early point during evolution; evidenced by the fact that
biomolecules implicated in stress response exhibit remarkable similarity across the animal
kingdom. Welch, W. J., *et al.*, *The Stress Response and the Immune System. Inflammation:*
Basic Principles and Clinical Correlates, Raven Press. Gallin, J.I., *et al.*, Eds., Second Edition,
41:841 (1992).

20 Lymphocyte activation, homing, resistance to target cell lysis, tumor antigenicity,
regulation of proto-oncogene transcription, and immune surveillance are examples of
immunologic functions that appear to be mediated or modulated by stress activated signal
transduction molecules. Siegelman, M., *et al.*, *Science*, 231:823 (1986); Kusher, D.I., *et al.*,
J. Immunol., 145:2925 (1990); Ullrich, S.J., *et al.*, PNAS, 83:3121 (1986); Colotta, F., *et al.*,
25 Biochem. Biophys. Res. Commun., 168:1013 (1990); Haire, R.N., *et al.*, J. Cell Biol,
106:883 (1988); Born, W., *et al.*, Immunol. T., 11:40 (1990). The number of preactivated
and MHC class II-restricted autoreactive T-lymphocytes in peripheral blood of patients with
rheumatoid arthritis, for example, dramatically increases relative to the levels in healthy
individuals. Similarly, peripheral blood T-lymphocytes from patients with inflammatory
30 arthritis proliferate strongly in the absence of exogenous antigen or mitogen. Welch, W. J., *et al.*,
The Stress Response and the Immune System. Inflammation: Basic Principles and Clinical

Correlates. Raven Press, Gallin, J.I., *et al.*, Eds., Second Edition, Chapter 41, 841 (1992).

Moreover, synovitis has been shown to result in the generation of oxygen-derived free radicals that act to perpetuate tissue damage. Blake, D.R., *et al.*, Hypoxic-Reperfusion Injury in the Inflamed Human Joint. *Lancet*, 2:2889 (1989).

5 The control of hematopoiesis is a highly regulated process that responds to a number of physiological stimuli in the human body. Differentiation, proliferation, growth arrest, or apoptosis of blood cells depends on the presence of appropriate cytokines and their receptors, as well as the corresponding cellular signal transduction cascades. Hu, Mickey C.-T., *et al.*, *Genes & Development*, 10:2251(1996). Generation of mature leukocytes, for instance, is a
10 highly regulated process which responds to various environmental and physiological stimuli. Cytokines cause cell proliferation, differentiation or elimination, each of these processes being dependent on the presence of appropriate cytokine receptors and the corresponding signal transduction elements. Moreover, the stimulation of quiescent B- and T-lymphocytes occur *via* antigen receptors which exhibit remarkable homology to cytokine receptors. Grunicke,
15 Hans H., *Signal Transduction Mechanisms in Cancer*, Springer-Verlag (1995). See also, Suchard, S.J., *et al.*, *Mitogen-Activated Protein Kinase Activation During IgG-Dependent Phagocytosis in Human Neutrophils*, *J. Immunol.*, 158:4961 (1997).

Distinct signaling cassettes, each containing a central cascade of kinases, respond to a variety of positive and negative extracellular stimuli, lead to changes in transcription factor
20 activity and posttranslational protein modifications in mammalian cells. Kiefer, F., *et al.*, *EMBO*, Vol. 5, 24:7013 (1996). One such protein kinase cascade, known as the *mitogen-activated protein kinase* (MAPK) cascade, is activated as an early event in the response of leukocytes to various stimuli. Stimulation of this pathway has been observed during growth factor-induced DNA synthesis, differentiation, secretion, and metabolism. The MAPK
25 pathway has a critical role in the transduction of receptor-generated signals from the membrane to the cytoplasm and nucleus. Graves, J. D., *et al.*, *Protein Serine/Threonine Kinases of the MAPK Cascade*, *Annals New York Academy of Sciences*, 766:320 (1995). It has been established that sustained activation of the MAPK cascade is not only required, but it is sufficient to trigger the proliferation of some cells and the differentiation of others. Cohen,
30 P., *Dissection of Protein Kinase Cascades That Mediate Cellular Response to Cytokines and Cellular Stress*, *Advances in Pharmacology*. Academic Press. Hidaka, H., *et al.*, Eds., Vol. 36,

15 (1996); Marshall, C.J., Cell, 80:179 (1995). Several interdependent biochemical pathways are activated following either stimulation of resting T-lymphocytes through the antigen receptor or stimulation of activated T-lymphocytes through the interleukin-2 (IL-2) receptor. Many of the events that occur after the engagement of either of these receptors are

5 qualitatively similar, such as the activation of mitogen-activated protein kinase (MAPK) pathways and preexisting transcription factors, leading to the expression of specific growth-associated genes. *Symmetry of the Activation of Cyclin-dependent Kinases in Mitogen and Growth Factor-stimulated T Lymphocytes*, Jaime F. Modiano, et al., Annals New York Academy of Sciences, 766:134 (1995).

10 Recent evidence suggests that cellular response to stress is controlled primarily through events occurring at the plasma membrane, overlapping significantly with those important in initiating mitogenic responses. Exposure of cells to biological, chemical, or physical stress agents evokes a series of events leading to the activation of a wide group of genes including transcription factors as well as other gene products that are also rapidly and
15 highly induced in response to mitogenic stimulation. The mitogen-activated protein kinase (MAPK) pathway has been shown to be essential for the mitogenic response in many systems. See, e.g., Qin, Y. et al., J.Cancer Res.Clin.Oncol., 120:519 (1994). Moreover, due to the fact that most oncogenes encode growth factors, growth factor receptors, or elements of the intracellular postreceptor signal-transmission machinery, it is becoming increasingly apparent
20 that growth factor signal transduction pathways are subject to an elaborate network of positive and negative cross-regulatory inputs from other transformation-related pathways. Grunicke, Hans H., *Signal Transduction Mechanisms in Cancer*, Springer-Verlag (1995). The Hierarchical organization of the MAPK cascade makes integral protein kinase members particularly good targets for such "cross-talk". *Protein Serine/Threonine Kinases of the*
25 *MAPK Cascade*, J. D. Graves, et al., Annals New York Academy of Sciences, 766:320 (1995).

Initial triggers for inflammation include physical and chemical agents, bacterial and viral infections, as well as exposure to antigens, superantigens or allergens, all of which have the potential to generate Reactive Oxygen Species (ROS) and to thereby activate second
30 messenger signal transduction molecules. Storz, G., et al., *Transcriptional Regulators of Oxidative Stress-Inducible Genes in Prokaryotes and Eukaryotes*, in: Stress-Inducible Cellular

Responses, Feige, U., et al., Eds., Birkhauser Verlag (1996). Reactive oxygen radicals, via damage to many cellular components including DNA, can cause cell death or, if less severe, cell cycle arrest at growth-phase checkpoints. Stress damage not only activates checkpoint controls but also activates protein kinases, including the stress activated protein kinases (SAPKs), c-Raf-1 and ERKs, which are integral components of cytoplasmic signal transduction (MAPK) cascades. Pombo, C. M., et al., EMBO, Vol. 15, 17:4537 (1996); Russo, T., et al., J.Biol. Chem., 270:29386 (1995). Considering, *inter alia*, that stress has also been implicated in oxidant injury, atherosclerosis, neurogenerative processes, and aging, elucidation of the components of mammalian stress-induced pathways should provide more specific targets that can be exploited therapeutically. N. J. Holbrook, et al., *Stress Inducible Cellular Responses*, 273, U. Feige, et al., Eds., Birkhauser Verlag (1996).

Evidence has demonstrated that mitogen-activated protein kinase (MAPK) and stress activated protein kinase (SAPK) signal transduction pathways are responsible for triggering biological effects across a wide variety of pathophysiological conditions including conditions manifested by dysfunctional leukocytes, T-lymphocytes, acute and chronic inflammatory disease, auto-immune disorders, rheumatoid arthritis, osteoarthritis, transplant rejection, macrophage regulation, endothelial cell regulation, angiogenesis, atherosclerosis, fibroblasts regulation, pathological fibrosis, asthma, allergic response, ARDS, atheroma, osteoarthritis, heart failure, cancer, diabetes, obeisity, cachexia, Alzheimers disease, sepsis, and neurodegeneration. As MAP kinases play a central role in signaling events which mediate cellular response to *stress*, their inactivation is key to the attenuation of the response. N. J. Holbrook, et al., *Stress-Inducible Cellular Responses*, 273, Feige, U., et al., Eds., Birkhauser Verlag (1996).

Despite major efforts to develop new therapeutic approaches Adult Respiratory Distress Syndrome (ARDS) (acute pulmonary inflammation characterized by the massive generation of Reactive Oxygen Species (ROS) within the lung), for example, remains lethal for about 50% of affected patients. Polla, B.S., et al., *Stress Proteins in Inflammation*, in: *Stress Inducible Cellular Responses*, Feige, U., et al. Eds., Birkhauser Verlag (1996). Moreover, the chronic inflammatory disease, rheumatoid arthritis, for instance, is believed to be mediated by activated T-lymphocytes that infiltrate the synovial membrane and initiate a series of inflammatory processes. Panayi, G.S., et al., *The Importance of the T-Cell in*

Initiating and Maintaining the Chronic Synovitis of Rheumatoid Arthritis, *Arthritis Rheum.* 35:729 (1992). Accumulating evidence also indicates that the autoimmune disease multiple sclerosis (MS) is mediated by autoreactive T-lymphocytes. Stinissen, P., *et al.*, *Crit. Rev. Immunol.* 17(1):33 (1997). Autoreactive T-lymphocytes have been demonstrated to undergo
5 *in vivo* activation and clonal expansion in patients with MS. Zhang, J., *et al.*, *J. Mol. Med.*, 74(11):653 (1996). In diabetes mellitus, autoreactive T-lymphocytes systematically destroy pancreatic islet cells such that they prove incapable of producing insulin. Another propelling recent development in the implication of overactive T-cells is the recognition that a particular subset of T-lymphocytes appear to be a major culprit in asthma and other allergic diseases, by
10 responding with undue vigor to apparently harmless invaders (rates of asthma per capita in the developing world have increased dramatically in the last several decades; doubling in the U.S. since 1980). *New Clues to Asthma Therapies*: Vogel, G., *Science*, 276:1643 (1997).

Recently, much progress has been made in defining the signal transduction pathways mediating the cellular response to stress. Pombo, C.M., *et al.*, for instance, report the cloning
15 and characterization of a human Ste20-like oxidant stress response kinase, SOK-1. The kinase is positively regulated by phosphorylation and negatively regulated by its C-terminal non-catalytic region. Reported data suggests SOK-1 transduces signals in response to oxidative and environmental stress. *EMBO*, Vol. 15, 17:4537 (1996). Moreover, Schinkmann, K., *et al.*, recently reported the cloning and characterization of the human STE-
20 20-like kinase, mst-3. The mst-3 transcript is reported to be ubiquitously expressed. Mst-1 is furthermore reported to be positively regulated by autophosphorylation. *J. Biol. Chem.*, 272(45):28695 (1997). Other stress-activated protein kinase (SAPK), members of the MAPK family, have been shown to be activated *in situ* by inflammatory stimuli, including tumor-necrosis factor (TNF) and interleukin-1. Kyriakis, J.M., *et al.*, *Nature*, 369:156 (1994);
25 Dérjard, B., *et al.*, *Cell*, 76:025 (1994); Sánchez, I., *et al.*, *Nature*, 372:794 (1994). *See also*, Kiefer, F., *et al.*, *EMBO*, Vol. 5, 24:7013 (1996); Creasy, C.L., *et al.*, *J. Biol. Chem.*, 271: No. 35, 21049 (1996); Creasy, C.L., *et al.*, *Gene*, 167:303 (1995); Manser, E., *et al.*, *Nature*, 367:40 (1994); Hu, Mickey C.-T., *et al.*, *Genes & Development*, 10:2251 (1996); Katz, P., *et al.*, *J. Biol. Chem.*, (1994); Pombo, C.M., *et al.*, *Nature*, 377:750 (1995).
30 Integral members of cellular signaling pathways as targets for therapeutic development, for example, have been the subject to several reviews. *See, e.g.*, Levitzki, A., *Signal-*

Transduction Therapy: A Novel Approach to Disease Management, Eur. J. Biochem, 226:1 (1994); Powis G., *The Potential for Molecular Oncology to Define New Drug Targets*, in: New Molecular Targets for Cancer Chemotherapy, Workman, P., Kerr D.J., eds., CRC Press, Boca Raton FL (1994). As a result of the efforts of numerous laboratories, an impressive list
5 of remarkably specific inhibitors of kinases, for instance, has become available. See, e.g., Levitzki, A., *Tyrphostins: Tyrosine Kinase Blockers as Novel Antiproliferative Agents and Dissectors of Signal Transduction*, FASEB, 6:3275 (1992); Workman P., et al., *Discovery and Design of Inhibitors of Oncogenic Tyrosine Kinases*, in: *New Approaches in Cancer Pharmacology: Drug Design and Development*, Springer, Berlin 55 (1994).

10 A novel class of pyridinyl imidazoles, CSAIDS [SKB], for instance, have been developed, that inhibit the production of the cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF- α) in monocytes. The drug has been demonstrated to bind specifically to one protein in monocytes, termed CSBP (CSAID-binding protein), which has been isolated, cloned, and sequenced and demonstrated as a MAPK homolog. Lee, J.C., et al., *Differential*
15 *Effects of the Bicyclic Imidazoles on Cytokine Synthesis in Human Monocytes and Endothelial Cells*, Agents Actions, 41:C191 (1994); *A Protein Kinase Involved in the Regulation of Inflammatory Cytokine Biosynthesis*, Nature, 372:739 (1994). Moreover, as demonstrated by the identification of rapamycin as a *specific* inhibitor of the activation of p70 S6 kinase and the identification of compounds that inhibit the EGF receptor protein kinase very potently and
20 that block the activation of MAP kinase kinase have demonstrated that specific inhibitors of protein kinases can indeed be developed. Alessi, D., et al., *A Specific Inhibitor of the Activation of MAP Kinase Kinase-1 in vitro and in vivo*, J. Biol. Chem., 279:27489 (1995); Fry, D., et al., *A Specific Inhibitor of the Epidermal Growth Factor Receptor Tyrosine Kinase*, Science, 265:806 (1994); Cohen, P., *Dissection of Protein Kinase Cascades That Mediate*
25 *Cellular Response to Cytokines and Cellular Stress*, Intracellular Signal Transduction, Advances in Pharmacology, Hidaka, H., et al., Eds., Academic Press, 36:17 (1996).

Compounds which are able to modulate the activity of specific signal transduction molecules integral to specific intracellular pathways are expected to have significant potential for the ability to control or attenuate downstream physiological responses. Unfortunately, in
30 spite of the introduction of numerous new drugs during the last three decades, there is a need

for new, more efficient and less toxic compounds. Accordingly, the ability to identify such compounds is of paramount importance.

Summary of the Invention

The present invention is directed to an isolated and purified polynucleotide molecule
5 comprising a nucleic acid sequence which encodes a polypeptide of a signal transduction
kinase substantially as depicted in SEQ ID NO:3 or a pharmacologically active or biologically
active functional derivative thereof. Isolated and purified polynucleotides of the present
invention include but are not limited to SEQ ID NO:1 (novel human signal-transduction
kinase cDNA), SEQ ID NO:11 (murine variation), and SEQ ID NO:2 (novel human signal-
10 transduction kinase structural coding region).

Isolated and purified polynucleotides of the present invention include but are not
limited to sequences comprising SEQ ID NO:1 and SEQ ID NO:2.

The current invention is directed to a purified polypeptide comprising the amino acid
sequence substantially as depicted in SEQ ID NO:3.

15 A preferred embodiment of the invention is an isolated and purified biologically
effective polynucleotide molecule comprising an antisense nucleic acid sequence derived from
SEQ ID NO:1 which has the ability to modulate the transcription/translation of a nucleic acid
coding region of the signal transduction molecule of the present invention.

The present invention is also directed to an isolated and purified polynucleotide
20 molecule derived from SEQ ID NO:1 comprising a nucleic acid sequence which encodes a
biologically effective dominant negative mutant polypeptide substantially as depicted in SEQ
ID NO:3 which has the ability to modulate the biological activity and/or pharmacological
activity of the signal transduction biomolecule of the present invention.

A further preferred embodiment of the invention is an isolated and purified
25 biologically effective dominant negative mutant polypeptide derived from SEQ ID NO:3
which has the ability to modulate the biological activity and/or pharmacological activity of the
native signal transduction polypeptide of the present invention.

The invention is further directed to an expression vector for the expression of a signal
transduction polypeptide in a recombinant host cell, wherein said vector contains a
30 polynucleotide comprising a nucleic acid sequence which encodes a polypeptide having the

sequence substantially as depicted in SEQ ID NO:3 or a pharmacologically and/or biologically active or biologically effective derivative thereof.

The invention is further directed to an expression vector for the expression of a biologically effective polynucleotide molecule comprising an antisense nucleic acid sequence
5 derived from SEQ ID NO:1 which has the ability to modulate the transcription/translation of a nucleic acid coding region of the signal transduction molecule of the present invention.

Further the invention is directed to a host cell containing an expression vector for expression of a signal transduction polypeptide, wherein said vector contains a polynucleotide comprising a nucleic acid sequence which encodes the polypeptide of a signal transduction
10 peptide having the sequence substantially as depicted in SEQ ID NO:3 or a pharmacologically and/or biologically active or biologically effective derivative thereof.

The instant invention is further directed to a method of identifying compounds that modulate the biological activity and/or pharmacological activity of a signal transduction molecule, comprising:

- 15 (a) combining a candidate compound modulator of signal transduction activity with a polypeptide having the sequence substantially as depicted in SEQ ID NO:3, and
(b) measuring an effect of the candidate compound modulator on the biological and/or pharmacological activity of the polypeptide sequence substantially as depicted in SEQ ID NO:3.

20 The instant invention is further directed to a method of identifying compounds that modulate the biological and/or pharmacological activity of a signal transduction molecule, comprising:

- (a) combining a candidate compound modulator of signal transduction activity with a host-cell expressing a signal transduction polypeptide having the sequence substantially as
25 depicted in SEQ ID NO:3, and
(b) measuring an effect of the candidate compound modulator on the signal transduction polypeptide biological and/or pharmacological activity.

The present invention is also directed to a compound identified by means of one of the aforementioned methods, wherein said compound modulates the biological and/or
30 pharmacological activity of a signal transduction molecule.

Further, the invention is directed to a pharmaceutical composition comprising a compound identified by means of one of the aforementioned methods, wherein said compound modulates the biological and/or pharmacological activity of a signal transduction molecule.

5 Additionally, the invention is directed to a method of treatment of a patient in need of such treatment for a pathophysiological condition mediated by a signal-transduction molecule, comprising administration of an effective amount of a compound identified by means of one of the aforementioned methods, wherein said compound modulates the biological and/or pharmacological activity of a signal transduction molecule; or the administration of an
10 effective amount of a biologically effective dominant negative mutant substantially as depicted in SEQ ID NO:3 or a functional derivative thereof.

 Additionally, the invention is directed to a method of treatment of a patient in need of such treatment for a pathophysiological condition mediated by a signal-transduction molecule, comprising administering an effective amount of a biologically effective antisense nucleic
15 acid molecule derived from SEQ ID NO:1; or administering an effective amount of a nucleic acid which encodes a biologically effective dominant negative mutant substantially as depicted in SEQ ID NO:3 or a functional derivative thereof.

 The current invention is also drawn toward an antibody specific for a purified polypeptide comprising the amino acid sequence substantially as depicted in SEQ ID NO:3, as
20 well as a diagnostic composition for the identification of a polypeptide sequence comprising the amino acid sequence substantially as depicted in SEQ ID NO:3.

 The invention is also directed to PCR primers derived from SEQ ID NO:1 as well as to methods of making nucleic acid molecules substantially as depicted in SEQ ID NO:1 and SEQ ID NO:2.

25 **Brief Description of the Figures**

 Figure 1 displays SEQ ID NO:1 which is a 3201 base cDNA nucleic acid sequence which encodes the novel human signal transduction kinase polypeptide described herein.

 Figure 2 displays SEQ ID NO:2 which is the 1251 base translated structural region, ATG to TAA, of the cDNA nucleic acid sequence which encodes the novel human signal
30 transduction kinase polypeptide described herein.

Figure 3 displays SEQ ID NO:3 which is the 416 amino acid residue sequence of the novel human signal transduction kinase polypeptide described herein.

Figure 4 shows SEQ ID NO:4 which is the 426 amino acid residue sequence of SOK-1, a recently described human oxidant stress activated kinase. Pombo, C.M., *et al.*, EMBO, Vol. 15, 17:4537 (1996).

Figure 5 shows SEQ ID NO:5 which is the 431 amino acid residue sequence of mst-3, a recently described human signal transduction kinase. Schinkmann, K., *et al.*, J. Biol. Chem., 272(45):28695 (1997).

Figure 6 shows a comparison between the amino acid residue sequence of the novel human signal transduction kinase polypeptide described herein (SEQ ID NO:3) (designated TEN-1), and the murine variant of the signal transduction kinase polypeptide (SEQ ID NO:11) (designated vx21d04), and the amino acid residue sequences of the SOK-1 stress activated kinase (SEQ ID NO:4) and the amino acid residue sequences of the mst-3 signal transduction kinase (SEQ ID NO:5). Dashes represent gaps introduced to optimize the alignment. Sequences shown in this figure were produced using the multisequence alignment program of DNASTAR software (DNASTAR Inc, Madison WI).

Figure 7 displays SEQ ID NO:6 and SEQ ID NO:7 which are oligonucleotide PCR primers used to produce full-length nucleic acid sequence amplicon pertaining to the novel human kinase.

Figure 8 displays SEQ ID NO:8 which is a 1353 base PCR amplicon produced using the sense, SEQ ID NO:6, and antisense, SEQ ID NO:7, oligonucleotides.

Figure 9 displays SEQ ID NO:9 and SEQ ID NO:10 which are a set of oligonucleotide primers for the PCR production of a 289bp amplicon indicative of expression of the novel kinase.

Figure 10 shows Northern blot analysis wherein a primary transcript of approximately 3.5kb in length pertaining to the novel human signal transduction kinase. Prominent transcripts are predominant in immune tissues as well as from cancer cell-lines.

Figure 11 shows kinase assay data showing that GST-TEN1 is capable of autophosphorylation and substrate phosphorylation (myelin basic protein).

Figure 12 displays SEQ ID NO:11 which is a murine 416 amino acid residue variant embodiment of the signal transduction kinase polypeptide (SEQ ID NO:3) described herein.

-11-

Figure 13 displays SEQ ID NO:12 which is a 2028 base cDNA nucleic acid sequence which encodes the murine 416 amino acid residue variant embodiment of the signal transduction kinase polypeptide described herein. The 5' UTR and 3' UTR are shown in lower case and the open reading frame is shown in upper case. The initiation and stop codons are underlined.

Figure 14 illustrates the activation of NF κ B, AP-1, and SRF in 293 cells by the signal transduction kinase SEQ ID NO:3 (TEN1 designates the signal transduction kinase (SEQ ID NO:3)).

Figure 15 illustrates the activation of AP-1, and SRF in HeLa cells by the signal transduction kinase SEQ ID NO:3 (TEN1 designates the signal transduction kinase (SEQ ID NO:3)).

Figure 16 illustrates the effects of the expression of SEQ ID NO:1 antisense molecules on basal level and TNF α -mediated NF κ B activation in ECV304 cells.

Detailed Description of the Invention

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All publications and patents referred to herein are incorporated by reference.

Biological activity as used herein in reference to the signal transduction serine threonine protein kinase of the present invention refers to the ability of the biomolecule to transduce cellular signals including but not limited to the ability to autophosphorylate and/or to phosphorylate a substrate.

Pharmacological activity as used herein in reference to the signal transduction serine threonine protein kinase of the present invention refers to the ability to mediate any one or more of the physiological conditions including but not limited to cell differentiation, proliferation, oncogenic transformation, macrophage regulation, endothelial cell regulation, fibroblasts regulation, cytoskeletal structure, metastases, cell aggregation, cell motility, cytokinesis, acute and chronic inflammatory disease, auto-immune disorders, allergic response, secretion, apoptosis, neurological disorders, peripheral vascular disease, atherosclerosis, and asthma.

Dominant negative mutant as used herein refer to a nucleic acid coding region sequence which has been changed with regard to at least one position in the sequence, relative

-12-

to the corresponding wild type native version, preferably at a position which changes an amino acid residue position at an active site required for biological and/or pharmacological activity in the native peptide to thereby encode a mutant peptide. In the context of this specification active sites include, but are not limited to, a catalytic kinase domain, a CRIB domain or p21 binding domain, and an SH3 binding domain. Dominant negative mutant as used herein also, in the same manner, may be used to refer to a mutant peptide.

Biologically effective as used herein in reference to antisense nucleic acid molecules as well as dominant negative mutant nucleic acid coding regions and dominant negative mutant peptides refers to the ability of these molecules to modulate the biological activity and/or pharmacological activity of the novel signal transduction protein kinase of the present invention and/or transcription/translation of nucleic acid coding regions of the novel signal transduction protein kinase of the present invention.

Substantially as depicted as used herein refers to functional derivative proteins, and functional derivative nucleic acid sequences as defined herein that may have changes but perform substantially the same biochemical or pharmacological function in substantially the same way; however, 'substantially as depicted' as used herein also refers to biologically effective dominant negative mutants and is intended to encompass biologically effective antisense molecules as defined herein.

As used herein, a functional derivative of a biomolecule disclosed herein is an entity that possesses a functional biological activity and/or pharmacological activity as defined herein that is derived from SEQ ID NO:1 or SEQ ID NO:3, for example, truncated versions, versions having deletions, functional fragments, versions having substitutions, versions having insertions or extended ends, or biologically effective dominant negative mutants as well as biologically effective antisense molecules.

The term modulation is used herein to refer to the capacity to either enhance or inhibit a biological activity and/or pharmacological activity of a signal transduction molecule of the present invention or to the capacity to either enhance or inhibit a functional property of a nucleic acid coding region of the present invention. Modulate physiology as used herein refers to the biophysiological regulation of cells and/or tissue and the treatment of pathophysiological disorders related thereto.

-13-

Direct administration as used herein refers to the direct administration of nucleic acid molecules, peptides, or compounds which comprise embodiments and/or functional derivatives (e.g., SEQ ID NO:1, SEQ ID NO:3) of the present invention. Direct administration includes but is not limited to gene therapy.

5 Purified as used herein refers to molecules, either nucleic acid or amino acid sequences, that are removed from their natural environment and isolated or separated from at least one other component with which they are naturally associated.

Expression vector as used herein refers to nucleic acid vector constructions to direct the transcription of nucleic acid regions in host cells. Expression vectors include but are not
10 limited to plasmids, retroviral vectors, viral and synthetic vectors.

Transformed host cells as used herein refer to cells which harbor nucleic acids or functional derivatives of the present invention.

Signal transduction via kinases

A cascade signal transduction mechanism is essentially a conduit for the transmittal of
15 an external stimulus to the cell nucleus in order to trigger a distinct pattern of gene expression in response to the stimulation. The extracellular signal is amplified and *transduced* by a series of independent sequential covalent modifications, from the plasma membrane to the nucleus, *via* distinct phosphorylation steps of independently specific cognate cytosolic biomolecules. Protein phosphorylation is now acknowledged as the most important means of acute
20 regulation of protein function, signal transduction, and gene expression in eukaryotic cells. Intracellular biomolecule phosphorylation via specific kinases is responsible for *switching* of cellular activity from one state to another. It is the major mechanism by which cells respond to extracellular signals such as mitogenic stimulation; biological, chemical, and physical stress, hormones, and growth factors. Protein Phosphorylation, Hardie, D. G., Oxford Press
25 (1993).

The protein kinases are a large family of enzymes. Conserved structural motifs provide clear indications as to how the kinases transfer the γ -phosphate of a purine nucleotide triphosphate to the hydroxyl groups of their protein substrates. There are two main subdivisions within the superfamily: the protein-serine/threonine kinases and the protein-
30 tyrosine kinases. The kinase domains that define protein kinases contain 12 conserved subdomains (I-XII) that fold into a common catalytic core structure, as revealed by the 3-

dimensional structures of several enzymes. The central core of the catalytic domain, the region with greatest frequency of highly conserved residues, consists of subdomains VI through IX. The most striking indicator of amino acid specificity is found in subdomain VI, the consensus in this region is a strong indicator of serine/threonine specificity. See, e.g.,

- 5 Hanks, S.K., *et al.*, *The Protein Kinase Family: Conserved Features and Deduced Phylogeny of the Catalytic Domains*, Science, 241:42 (1988); Hanks, S.K., *et al.*, *The Eukaryotic Protein Kinase Superfamily: Kinase (Catalytic) Domain Structure and Classification*, FASEB J. Ser. Rev., 9:576 (1995).

- Protein kinases which have closely related catalytic domains, and thus define a family,
10 represent products of genes that have undergone relatively recent evolutionary divergence. Clustering appears to be of predictive value in the determination of the properties and function of novel protein kinases. Accordingly, members of a given family tend also to share related functions. This is manifest by similarities in overall structural topology, mode of regulation, and substrate specificity. See, generally, Hardie, D. G., *et al.*, *The Protein Kinase Factsbook*,
15 Academic Press, London (1995).

- Progress has been made by many labs in defining signaling pathways initiated by mitogenic stimuli. Blenis, J., *Signal Transduction via the MAP Kinases*, PNAS, 90:5889 (1993). The MAP kinase family of enzymes have been implicated as common and essential components of signaling pathways induced by diverse mitogenic stimuli. Once activated,
20 MAP kinases phosphorylate a number of substrates including transcription factors essential for triggering gene expression required for the growth response. Accordingly, the MAP kinases are considered to be potentially valuable pharmacological targets within the growth factor signaling pathways. Hidaka, H., *et al.*, *Intracellular Signal Transduction*, Advances in Pharmacology, Academic Press (1996).
- 25 Mitogen-activated protein kinases (MAPKs) and their upstream regulatory kinases comprise functional units that couple upstream input signals to a variety of outputs. MAPK cascades have been remarkably conserved in evolution. The core of these cascades is a three-tiered module consisting of an MAPK-extracellular signal-regulated kinase kinase (an MEKK), an MEK and an MAPK or extracellular signal-regulated kinase (ERK). The defining
30 characteristic of these modules is the MAPK itself. The classical pathway, known as the extracellular signal-regulated kinase pathway (ERK), is activated by mitogens and growth

factors. ERK has a regulatory kinase, MAPK kinase or MEK, necessary for activation. This enzyme is in turn regulated by another MAPKK kinase known as Raf. Analogous with the classical MAPK module are two other modules which are activated by cytokines and cellular stresses and which have become known as the stress kinase pathways. The defining MAPKs
5 of these pathways are JNK (SAPK) and P38. JNK is activated by the upstream SEK-1 (MKK4) which is activated by MEKK1 or MLK3 whereas P38 is activated by MKK3 and MKK6.

In yeast the MAPK modules operate in a linear manner linking extracellular signals to functional response, whereas in mammalian cells 'cross-talk' between modules may be
10 obligatory in some cases (eg. IL-2 production by T-lymphocytes). Phosphorylation of transcriptional factors (eg. AP-1, NF-kB, ELK-1, ATF-2) by the terminal MAPKs serve to regulate expression of key inflammatory genes. Differences in the expression of the various kinases between cell types and in response to processes in disease will have major impact on how cells respond to extracellular stimuli under physiological and pathological conditions. It
15 is for these reasons that there is very likely to be selectivity for specific inhibitors of these different kinases for their associated physiological role as well as opportunities for therapeutic intervention.

Phosphorylation of transcriptional factors (eg. AP-1, NF-kB, ELK-1, ATF-2) by the terminal MAPKs serve to regulate expression of key inflammatory genes. Differences in the
20 expression of the various kinases between cell types in response to processes in disease will have major impact on how cells respond to extracellular stimuli under physiological and pathological conditions. This should provide opportunities for therapeutic intervention.

Studies of the budding and fission yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, have been particularly fruitful in the recognition of protein
25 kinases. Hanks, S. K., *et al.*, *The Eukaryotic Protein Kinase Superfamily*, FASEB Ser. Rev., 9:576 (1995). Signal transduction pathways connecting cell surface receptors with each member of the MAPK superfamily in mammalian cells are remarkably similar to those of the budding yeast *Saccharomyces cerevisiae*, in which genetic studies have shown parallel signaling cascades leading to the activation of at least three distinct MAPK-related kinases.
30 Hu, Mickey C.-T., *et al.*, *Genes & Development*, 10:2251(1996); *See, e.g.*, Herskowitz, I., *et al.*, *Cell*, 80:187 (1995).

A component of the pheromone-response pathway in budding yeast, Ste20, represented the first identified member of a new family of serine/threonine protein kinases. Leberer, E., *et al.*, EMBO, 11:4815 (1992); Ramer, S. W., *et al.*, PNAS, 90:452 (1993). Several mammalian homologs to Ste20 have since been identified, including MST1 (Creasy, C.L., *et al.*, J. Biol. Chem., 271: No. 35, 21049 (1996)), MST2 (Gene, 167:303 (1995)), HPK1 (Kiefer, F., *et al.*, EMBO, Vol. 5, 24:7013 (1996)). Recently, mammalian Ste20-like kinases, including p21-activated protein kinase (PAK1) (Manser, E., *et al.*, Nature, 367:40 (1994)) and germinal center kinase (GC kinase) (Katz, P., *et al.*, J. Biol. Chem., (1994)), have been shown to be capable of activating mammalian MAPK cascades. Pombo, C.M., *et al.*, EMBO, Vol. 15, 17:4537 (1996). See also, U.S. Patent No. 5,605,825, Human PAK65, issued Feb. 25, 1997. Methods described in U.S. 5,605,825 are herein incorporated herein by reference.

The MAP kinases require activation by a MAPK/ERK activating kinase (MEK). The dual-specificity kinase is capable of phosphorylating both tyrosine and serine/threonine residues in proteins. The proto-oncogene c-Raf-1, for instance, has been shown to encode a protein acting as a MEK kinase and the pathway Raf → MEK → MAPK is now well established as a major signal transduction pathway for growth factors. Activated MAPK undergoes a translocation to the nucleus where it can directly phosphorylate and activate a variety of transcription factors including c-Myc, C/EBPβ, p62^{TCF}/Elk-1, ATF-2 and c-Jun. Grunicke, Hans H., *Signal Transduction Mechanisms in Cancer*, Springer-Verlag (1995).

The MAP kinase (MAPK) cascade is critical in mediating several intracellular actions. In one pathway a series of protein-protein interactions is triggered at the plasma membrane that culminate in activation of the GTP-binding protein Ras. GTP-Ras then interacts with the protein kinase Raf, recruiting it to the plasma membrane where it is activated. The activation of Raf is followed by the sequential activation of three additional kinases: MAP kinase kinase (MAPKK), MAPK, and MAPK-activated protein (MAPKAP) kinase-1. The activation of MAPK and MAPKAP kinase-1 leads to their translocation from the cytosol to the nucleus where they regulate the activities of transcription factors. Cohen, P., *Dissection of Protein Kinase Cascades That Mediate Cellular Response to Cytokines and Cellular Stress*, Advances in Pharmacology, Academic Press, Hidaka, H., *et al.*, Eds., Vol. 36, 15 (1996); Marshall, C.J., Cell, 80:179 (1995).

Recent evidence suggests that cellular response to stress is controlled primarily through events occurring at the plasma membrane, overlapping significantly with those important in initiating mitogenic responses. Exposure of cells to stress agents, as mentioned *supra*, evokes a series of events leading to the activation of a wide group of genes including transcription factors as well as other gene products that are also rapidly and highly induced in response to mitogenic stimulation. Pathways which are involved in mediating these cellular responses rely on the activation of mitogen-activated protein kinases (MAPK) which include extracellular signal-regulated kinases (ERK), stress activated protein kinases (SAPK), c-Jun N-terminal kinases (JNK), and p38/PK/CSBP kinases. These kinases play a key role in the activation of transcription factors and other regulatory proteins involved in activating gene expression. Phosphorylation enhances complex formation and the serum response element located in the promoters of stress response genes such as *c-fos*. Other regulated proteins include p90^{RSK}, activating transcription factor-2 (ATF-2, a cAMP response element-binding protein), NF-IL6 (nuclear factor for the activation of Interleukin 6) and c-Myc. Davis, R.J., *The Mitogen-Activated Protein Kinase Signal Transduction Pathway*, J. Biol. Chem., 268:1553 (1993); N. J. Holbrook, *et al.*, *Stress Inducible Cellular Responses*, 273, in: *Stress-Inducible Cellular Responses*, Feige, U., *et al.*, Eds., Birkhauser Verlag (1996). The mitogen-activated protein kinase (MAPK) pathway has been shown to be essential for the mitogenic response in many systems. *See, e.g.*, Qin, Y. *et al.*, J.Cancer Res.Clin.Oncol., 120:519 (1994). Moreover, due to the fact that most oncogenes encode growth factors, growth factor receptors, or elements of the intracellular postreceptor signal-transmission machinery, it is becoming increasingly apparent that growth factor signal transduction pathways are subject to an elaborate network of positive and negative cross-regulatory inputs from other transformation-related pathways. Grunicke, Hans H., *Signal Transduction Mechanisms in Cancer*, Springer-Verlag (1995). The Hierarchical organization of the MAPK cascade makes integral protein kinase members particularly good targets for such "cross-talk". *Protein Serine/Threonine Kinases of the MAPK Cascade*, J. D. Graves, *et al.*, Annals New York Academy of Sciences, 766:320 (1995).

Initial triggers for inflammation include physical and chemical agents, bacterial and viral infections, as well as exposure to antigens, superantigens or allergens, all of which have the potential to generate Reactive Oxygen Species (ROS) and to thereby activate second

messenger signal transduction molecules. Reactive oxygen species are generated from molecular oxygen and include the free radicals superoxide ($\cdot\text{O}_2^-$), hydroxyl ($\cdot\text{OH}$) and nitric oxide ($\text{NO}\cdot$), as well as non-radical intermediates such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\text{O}_2$). During normal cellular respiration, ROS are constantly produced at low rate in both eukaryotes and prokaryotes. At these low concentrations, ROS can act as second messengers, stimulate cell proliferation, and act as mediators for cell activation. However, during phagocytosis, infection or inflammation, ROS can accumulate to toxic levels which leads to oxidative stress, and may damage almost all cellular components. All organisms have mechanisms to detoxify the oxidants or to repair the damage caused by ROS, including superoxide dismutases, catalases, peroxidases, glutathione, thioredoxin, and heat shock proteins. The expression of the genes coding these proteins (oxidative stress genes) is induced by changes in the concentrations of ROS, suggesting that cells have developed mechanisms to sense the ROS. Storz, G., *et al.*, *Transcriptional Regulators of Oxidative Stress-Inducible Genes in Prokaryotes and Eukaryotes*, in: Stress-Inducible Cellular Responses, Feige, U., *et al.*, Eds., Birkhauser Verlag (1996).

Isozymes generally

The study of isozymes has grown into one of fundamental significance in the investigation of the molecular basis of cellular differentiation and morphogenesis. Isozymes represent a type of regulation accomplished through the participation of multiple forms of a given enzyme or enzyme subunits that occur within an organism, in different cell types, or even within a single cell. In many instances all forms of the particular enzyme catalyze the same overall reaction, e.g., phosphorylation, but differ in their dependence on substrate, substrate concentration, activators, cofactors, and cellular conditions. The relative proportions of isozymes in particular tissues is particularly important in the diagnosis of disease. Isozymes are now known for a great many different enzymes. Many allosteric enzymes occur as two or more isozymes that vary in sensitivity to their allosteric modulators. Different isozymes have accordingly evolved as mechanistic response to biochemical conditions - their presence therefore is a reliable factor in the indication of precise normal physiological or, in contrast, pathophysiological conditions.

SOK-1

Pombo *et al.* recently reported the cloning and characterization of human Ste20/oxidant stress response kinase, SOK-1 which belongs to the Sps1/GC kinase group of Ste20-like kinases with N-terminal catalytic domains. The kinase is positively regulated by 5 phosphorylation and negatively regulated by its C-terminal non-catalytic region. SOK-1 is activated relatively specifically by oxidant stress. Reported data places SOK-1 in a stress response pathway and suggest it resembles in function yeast Ste20s which transduce signals in response to environmental stress. EMBO, Vol. 15, 17:4537 (1996). The open reading frame is reported to encode a protein of 426 amino acids and has a predicted M_r of 48,041 Da. The 10 kinase domain is located in the N-terminal half of the protein. The reported peptide contains all 11 subdomains of serine/threonine kinases. Ste20 related stress-activated kinases are evidenced to be in proximity to the membrane in the signaling cascade and therefore are able to provide greater target opportunity for selective modulation of signal transduction.

MST-3

15 Schinkmann *et al.* recently reported the cloning and characterization of the human STE-20-like kinase, mst-3. The mst-3 transcript is reported to be ubiquitously expressed, is most closely related to SOK-1, and is furthermore reported to be positively regulated by autophosphorylation. A cDNA molecule composed of 1976 bases has been described as well as an open reading frame which encodes a 431 amino acid peptide (SEQ ID NO:5) having a 20 molecular mass of 48kDa. The mst-3 kinase is reported to consist of an N-terminal kinase domain and a 142-amino acid C-terminal regulatory domain. The mst-3 molecule shares 68.8% overall amino acid homology with SOK-1. J. Biol. Chem., 272(45):28695 (1997).

Novel human signal transduction serine/threonine kinase

A novel human signal transduction serine/threonine protein kinase molecule, as well as 25 example nucleic acid sequences which encode therefor, are herein described.

A cDNA sequence is provided, (SEQ ID NO:1) FIG.1, which comprises the structural coding region of the native human signal transduction kinase (SEQ ID NO:2) FIG.2. The 3201 bp SEQ ID NO:1 contiguous cDNA sequence contains a 1251 bp open reading frame (ORF) with a Kozak initiation sequence at the start methionine. FIG.2 (SEQ ID NO:2), shows 30 the 1251 base open reading frame including the stop codon. The native human homolog of the Ste20-like signal transduction kinase (SEQ ID NO:3) is shown in FIG.3. The 416 amino

acid residue sequence of the novel protein contains all eleven (11) subdomains found in eukaryotic protein kinases including Ste20-like kinases. SEQ ID NO:3 has 65.7 and 71 percent homology to the Ste20/oxidant stress response kinase-1 (SOK-1) (SEQ ID NO:4) described by Pombo *et al.*, and to the mammalian Ste20-like kinase (MST-3) (SEQ ID NO:5) described by Schinkmann *et al.*, respectively (FIG.6 shows a comparison). EMBO, Vol. 15, 17:4537 (1996); J. Biol. Chem., 272(45):28695 (1997). The novel kinase has a predicted molecular weight of 46527.83 daltons, an isoelectric point of 5.097, and a net charge of -13 at pH 7.0.

Mitogen-activated protein kinase cascades have been remarkably conserved in evolution. FIG.4, for example, shows the 426 amino acid residue sequence of SOK-1 (SEQ ID NO:4) which is the recently described human MAPK-pathway oxidant (stress) activated kinase. SOK-1 has been characterized as a human homolog of the MAPK-pathway yeast stress-activated kinase Ste20. Pombo, C.M., *et al.*, EMBO, Vol. 15, 17:4537 (1996). Pombo *et al.* reported SEQ ID NO:4 as a human Ste20/oxidant stress response kinase. Ste20 related stress-activated kinases, via evidentiary characterization, appear to be close to the plasma membrane in the signaling cascade and therefore may have significant potential to provide greater target opportunity for selective modulation of signal transduction. EMBO, Vol. 15, 17:4537 (1996).

Some features of the cDNA SEQ ID NO:1 are for instance: bases 1-181 represent the 5' UTR, bases 176-185 represent the Kozak sequence, bases 182-1432 represent CDS, bases 1433-3201 represent 3' UTR, bases 3194-3199 represent the poly adenylation signal.

Some features of the 416 residue polypeptide (SEQ ID NO:3) are for instance: Molecular Weight 46527.83 Daltons, 51 Strongly Basic(+) Amino Acids (K,R), 65 Strongly Acidic(-) Amino Acids (D,E), 138 Hydrophobic Amino Acids (A,I,L,F,W,V), 103 Polar Amino Acids (N,C,Q,S,T,Y). Isoelectric Point = 5.097, Charge at PH 7.0 = -13.017.

Eukaryotic protein kinases each contain a conserved catalytic domain region of 250-300 amino acid residues which is responsible for the phosphotransferase activity of the enzyme. The catalytic domain of the novel signal transduction kinase described herein (SEQ ID NO:3) has 89 and 87.5 percent similarity, respectively, with the catalytic domain of mst-3 (SEQ ID NO:5) and SOK-1 (SEQ ID NO:4). Catalytic domains can be further divided into 12 subdomains defined by strings of invariant and conserved residues. Residue positions 31-38

-21-

of the novel signal transduction kinase, for instance, correspond to the consensus kinase subdomain I GxGxxGxV. Subdomain II is involved in the phosphotransfer reaction and identified by an invariant lysine in the tripeptide sequence AxK. With regard to the novel kinase SEQ ID NO:3, subdomain II is found in residues 51-53 (AIK). Subdomains VI
5 through IX, characterized by a large number of highly conserved residues, form the central core of catalytic activity. SEQ ID NO:3 comprises the invariant or nearly invariant residues Asp¹⁴⁴ and Asn¹⁴⁹ in subdomain VI and Asp¹⁶², Phe¹⁶³, and Gly¹⁶⁴ in subdomain VII; all of which have been implicated in ATP binding. Region VIB contains the consensus sequence HRDLxxxN, with D being the invariant Asp¹⁴⁴. SEQ ID NO:3 in this region is HRDIKAAN
10 (wherein the substitution of Ile for Leu is conservative). Moreover, the novel signal transduction kinase contains the conserved DFG of subdomain VII. The Asp functions to orient the γ -phosphate of the ATP for transfer. Subdomain VIII of SEQ ID NO:3 contains the highly conserved APE sequence, with the Glu corresponding to the invariant Glu¹⁸⁹. This subdomain is thought to play a critical role in the recognition of substrate binding.

15 Additionally, many kinases are known to be activated by phosphorylation of residues in subdomain VIII. The sequence DxWS/AxG of subdomain IX is represented by amino acid positions 201-206 of SEQ ID NO:3 (DIWSLG). This region forms a large α -helix and the initial Asp of the consensus sequence serves to stabilize the catalytic loop by hydrogen bonding.

20 **Expression in immune tissues**

Northern blot analysis identifies a primary transcript pertaining to the novel signal transduction kinase of approximately 3.5kb in length. Prominent transcripts are apparent primarily in immune tissues (expressed at a particularly high level in tissues of the human immune system, i.e., lymph node, peripheral blood leukocytes, spleen, fetal liver, bone
25 marrow, thymus, and placenta) as well as from placenta and cancer-cell lines. See, FIG.10.; EXAMPLE V. Expression of the novel kinase appears to be in sharp contrast to the mst-3 transcript which is reported to be ubiquitously expressed. J. Biol. Chem., 272(45):28695 (1997).

Activation of transcription factors by TEN-1 kinase

The signal transduction kinase (SEQ ID NO:3) is demonstrated herein to activate NFκB, AP1, and SRF when overexpressed in cell lines (e.g., HEK293, HeLa). In addition, expression of an antisense cDNA has been shown to inhibit both basal and TNFα-mediated
5 activation of NFκB in ECV304 cells. Shown in Example XIV is the general format of the transcription activation reporter assays used to determine if SEQ ID NO:3 activates specific transactors. The PathDetect™ System (including reporter constructs and positive control constructs) was used to perform these assays. Stratagene, La Jolla, CA. All assays were performed in triplicate and repeated several separate times.

10 The signal transduction kinase (SEQ ID NO:3) activates NFκB, AP1, and SRF when overexpressed in HEK293 and HeLa cells. In addition, SEQ ID NO:1 anti-sense cDNA expression in ECV304 cells is capable of inhibiting both basal and TNFα stimulated inductions of NFκB by approximately 66% and 50%, respectively. SEQ ID NO:3 is able to activate transcription factors (e.g. NFκB, AP1) *in vivo* that are known to initiate transcription
15 of numerous inflammatory genes including pro-inflammatory cytokines, chemokines, inflammatory enzymes, adhesion molecules, and inflammatory receptors. In addition, NFκB synergistically interacts with AP1 to promote gene transcription. The co-operativity between NFκB and other transcription factors activated by inflammatory signals underlie the specificity of the inflammatory response in many different diseases.

20 Figure 14 illustrates the activation of NFκB, AP-1, and SRF in 293 cells by the signal transduction kinase SEQ ID NO:3 (TEN1 designates the signal transduction kinase (SEQ ID NO:3)). MEKK is a known activator of NFκB and is supplied as part of the Stratagene PathDetect™ System. Figure 15 illustrates the activation of AP-1, and SRF in HeLa cells by the signal transduction kinase SEQ ID NO:3 (TEN1 designates the signal transduction kinase
25 (SEQ ID NO:3)). MEKK is a known activator of AP-1 and is supplied as part of the Stratagene PathDetect™ System. PKA (protein kinase A) is a known activator of SRF and is supplied as part of the Stratagene PathDetect™ System. Figure 15 illustrates the effects of the expression of SEQ ID NO:1 antisense molecules on basal level and TNF α-mediated NFκB activation in ECV304 cells.

Variations

The present invention relates to nucleic acid (e.g., SEQ ID NO:1 and SEQ ID NO:2) and amino acid sequences (e.g., SEQ ID NO:3) of the novel human signal transduction kinase and variations thereof as well as functional derivatives and to the use of these sequences to
5 identify compounds that modulate the biological and/or pharmacological activity of the signal transduction molecule. Further, the invention relates to biologically effective antisense nucleic acid molecules as well as nucleic acids which encode biologically effective dominant negative mutant versions of the signal transduction kinase as well as biologically effective dominant negative mutant versions of the novel peptide biomolecule. The present invention
10 also provides a method of treatment for a patient in need of such treatment, *videlicet* for a patient who suffers a pathological condition mediated by the SEQ ID NO:3 signal transduction kinase, comprising administering an effective amount of a biologically effective antisense nucleic acid molecule derived from SEQ ID NO:1; or administering an effective amount of a nucleic acid which encodes a biologically effective dominant negative mutant
15 version of the signal transduction kinase; or administering a compound that modulates the biological and/or pharmacological activity of SEQ ID NO:3 which was identified by a method described herein.

The present invention also encompasses variants of the human signal-transduction kinase molecule SEQ ID NO:3. A preferred variant substantially as depicted in SEQ ID
20 NO:3, for instance, is one having at least 80% amino acid sequence similarity; a more preferred variant is one having at least 90% amino acid sequence similarity; and a most preferred variant is one having at least 95% amino acid sequence similarity to the kinase molecule amino acid sequence (SEQ ID NO:3) or a biologically active fragment thereof.

An embodiment of the current invention is set forth herein as SEQ ID NO:11 which is
25 a murine homolog of SEQ ID NO:3 having 98.1% amino acid sequence homology to the kinase molecule amino acid sequence (SEQ ID NO:3). *See* FIG.12.

A dominant negative/inactive kinase mutant, for example, can readily be produced with the tools provided herein to further produce one embodiment exemplified herein by changing the lysine at SEQ ID NO:3 position 53 to an arginine. *See* Example XII. Dominant
30 negative mutant versions of SEQ ID NO:3 may be delivered via gene therapy to treat

pathophysiological conditions otherwise that may be addressed by treatment with compounds which would antagonize the biological activity of SEQ ID NO:3 *in vivo*.

Variants including 'substantially as depicted' sequence embodiments presented herein include antisense molecules and biologically effective dominant negative mutants.

5 A variant of the human kinase molecule of the present invention substantially as depicted may have an amino acid sequence that is different by one or more amino acid substitutions. The variant may have conservative changes, wherein a substituted amine acid has similar structural or chemical properties, for example, the replacement of leucine with isoleucine. A variant may have nonconservative changes, e.g., replacement of a glycine with a
10 tryptophan. Similar minor variations may also include amine acid deletions or insertions, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTar software.

Polynucleotide sequences which encode the human signal-transduction molecule SEQ
15 ID NO:3 or a functional derivative thereof may be used in accordance with the present invention which comprise deletions, insertions and/or substitutions of the SEQ ID NO:2 nucleic acid sequence. Biologically active variants of the kinase molecule of the present invention may also be comprised of deletions, insertions or substitutions of SEQ ID NO:3 amino acid residues. A purified polynucleotide comprising a nucleic acid sequence encoding
20 the polypeptide having the sequence substantially as depicted in SEQ ID NO:3 or a biologically active and/or pharmacologically active fragment thereof is a particularly preferred embodiment of the present invention. *Biologically effective* antisense molecules as well as nucleic acids which encode biologically effective dominant negative mutant versions of SEQ ID NO:3, or derivatives thereof, are preferred embodiments of the present invention.

25 Amino acid substitutions of SEQ ID NO:3 may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as biological activity and/or pharmacological activity of the molecule is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine;
30 and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine

phenylalanine, and tyrosine. However, in the construction of biologically effective dominant negative mutants at least one amino acid residue position at an active site required for biological and/or pharmacological activity in the native peptide is changed to produce an agent or entity having reduced activity or which is devoid of detectable native wild type
5 activity.

Suitable substitutions of amino acids include the use of a chemically derivatized residue in place of a non-derivatized residue. D-isomers as well as other known derivatives may also be substituted for the naturally occurring amino acids. See, e.g., U.S. Patent No. 5,652,369, *Amino Acid Derivatives*, issued July 29, 1997. Example substitutions are set forth
10 in TABLE 1 as follows:

TABLE 1

Original residue	Example conservative substitutions
Ala (A)	Gly; Ser; Val; Leu; Ile; Pro
Arg (R)	Lys; His; Gln; Asn
Asn (N)	Gln; His; Lys; Arg
Asp (D)	Glu
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
Gly (G)	Ala; Pro
His (H)	Asn; Gln; Arg; Lys
Ile (I)	Leu; Val; Met; Ala; Phe
Leu (L)	Ile; Val; Met; Ala; Phe
Lys (K)	Arg; Gln; His; Asn
Met (M)	Leu; Tyr; Ile; Phe
Phe (F)	Met; Leu; Tyr; Val; Ile; Ala
Pro (P)	Ala; Gly
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr; Phe

Tyr (Y)	Trp; Phe; Thr; Ser
Val (V)	Ile; Leu; Met; Phe; Ala

The nucleotide sequences of the present invention may also be engineered in order to alter a coding sequence for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, 5 mutations may be introduced using techniques which are well known in the art, eg, site-directed mutagenesis to insert new restriction sites, to alter glycosylation patterns, to change codon preference, etc.

Included within the scope of the present invention are alleles of the human signal-transduction kinase molecule of the present invention. As used herein, an "allele" or "allelic 10 sequence" is an alternative form of the kinase molecule described herein. Alleles result from nucleic acid mutations and mRNA splice-variants which produce polypeptides whose structure or function may or may not be altered. Any given gene may have none, one or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions or substitutions of amino acids. Each of these types of changes 15 may occur alone, or in combination with the others, one or more times in a given sequence.

The present invention relates, in part, to the inclusion of the polynucleotide encoding the novel human signal-transduction kinase molecule in an expression vector which can be used to transform host cells or organisms. Such transgenic hosts are useful for the production of the protein kinase.

20 The nucleic acid sequence also provides for the design of antisense molecules useful in downregulating, diminishing, or eliminating expression of the genomic nucleotide sequence in cells including leukocytes, endothelial cells, and tumor or cancer cells.

The human signal-transduction kinase molecule of the present invention can also be used in screening assays to identify antagonists or inhibitors which bind, emulate substrate, or 25 otherwise inactivate or compete with the biomolecule. The novel kinase can also be used in screening assays to identify agonists which induce the production of or prolong the lifespan of the molecule *in vivo* or *in vitro*.

The invention also relates to pharmaceutical compounds and compositions comprising the kinase molecule substantially as depicted in SEQ ID NO:3, or fragments thereof, antisense

molecules capable of disrupting expression of the naturally occurring gene, and agonists, antibodies, antagonists or inhibitors of the native signal-transduction kinase. These compositions are useful for the prevention or treatment of conditions associated with abnormal expression of the signal transduction molecule such as described *infra*.

5 **Pharmacological significance**

Compounds which are able to modulate the activity of specific signal transduction molecules integral to specific intracellular pathways are expected to have significant potential for the ability to control or attenuate downstream physiological responses. Significant evidence has been provided that stress kinase activation pathways are responsible for
10 biological effects across a wide variety of disease areas. As MAP kinases play a central role in signaling events which mediate cellular response to stress; compounds which modulate or inactivate specific integral signal transduction molecules, i.e., the novel human stress-activated serine/threonine signal transduction kinase molecule described herein. SEQ ID NO:3, and nucleic acid sequences coding therefor, e.g. SEQ ID NO:1 and SEQ ID NO:2, have
15 significant potential for the ability to attenuate pathophysiological responses. Accordingly, the ability to screen for antagonists and agonists which modulate the activity of the native human stress-activated serine/threonine signal transduction kinase molecule described herein is significantly valuable toward the identification and development of therapeutic agents.

Potential diagnostic and therapeutic applications are readily apparent for modulators of
20 the human Ste20-like signal transduction serine/threonine kinase described herein. Areas which are common to disease particularly in need of therapeutic intervention include but are not limited to pathophysiological disorders manifested by dysfunctional leukocytes, T-cell activation, acute and chronic inflammatory disease, auto-immune disorders, rheumatoid arthritis, osteoarthritis, transplant rejection, macrophage regulation, endothelial cell
25 regulation, angiogenesis, atherosclerosis, fibroblasts regulation, pathological fibrosis, asthma, allergic response, ARDS, atheroma, osteoarthritis, heart failure, cancer, diabetes, obesity, cachexia, Alzheimers, sepsis, neurodegeneration, and related disorders.

Oxidants, mechanical stress, UV irradiation, and immunologic mediators lead to the activation of proinflammatory gene products, apoptosis-related genes, and acute phase
30 response genes. These responses are the underlying causes of vascular injury and inflammation. In view of the recent literature, it is generally accepted that MAPK pathways

including the SAPK/JNK and p38 kinase pathways, for example, are central components in the response of cells to chemical, mechanical and proinflammatory assaults. With such a prominent role in cell activation, stress kinases are likely to play an important role in disease areas including, but not limited to, stroke (ischemia reperfusion), ARDS, sepsis,

5 neurodegeneration, and inflammation. During reperfusion of ischemic tissue, for instance, there is a marked increase in stress activated protein kinase activity leading to c-Jun/ATF-2 activation to enhance gene transcription leading to either apoptosis or differentiation and cell repair. In the inflammatory response, TNF- α and IL-1 β activate p38, which triggers production of more TNF and IL-1, which amplifies the inflammatory response. This process
10 is thought to play a role in both septic shock and formation of the atherosclerotic plaque.

SAPKs are also thought to play a role in the induction of E-selectin expression in endothelial cells and matrix metalloproteinases in inflammatory cells, indicating a role for SAPKs in postischemic injury and tissue remodeling. Moreover, SAPK-p46 β_1 , a brain-specific kinase, colocalizes with the prominent Alzheimer's disease marker, ALZ-50, suggesting that the
15 proline-directed hyperphosphorylation of tau protein is catalyzed by this kinase.

The signal-transduction kinase homolog described herein (SEQ ID NO:3) is believed to transduce cellular response to stressors leading to the activation of proinflammatory gene products. The deleterious effects of the mediators of inflammation, including ROS and cytokines, open new avenues for the development of original anti-inflammatory therapies. As
20 MAP kinases play a central role in signaling events which mediate cellular response to stress, their inactivation or antagonization is key to the attenuation of the response. Clear evidence has been shown, for instance, that ERK and JNK pathways are strongly linked to IL-2 production. ERK and JNK pathways are clearly established to be required for full T-lymphocyte activation leading to IL-2 gene transcription and T-cell proliferation. Interruption
25 of the signaling process by selective kinase inhibition is therefore expected to reduce IL-2 production and T-lymphocyte proliferation which would be therapeutically beneficial in chronic inflammatory diseases. MAPKs including stress activated kinases are expected to play key roles in the pathology of autoimmune diseases including but not limited to rheumatoid arthritis (RA), osteoarthritis (OA), and multiple sclerosis (MS). Accordingly, the
30 novel human serine/threonine stress-activated kinase signal transduction molecule described herein (SEQ ID NO:3), and nucleic acid sequences coding therefor, e.g. SEQ ID NO:1 and

SEQ ID NO:2, have significant potential as a specific targets that can be exploited
diagnostically and therapeutically for the control of dysfunctional leukocytes, including but
not limited to dysfunctional T-lymphocytes, and in the treatment of autoimmune disease
including rheumatoid arthritis (RA) and osteoarthritis (OA), and in the study, diagnosis, and
5 treatment of acute and chronic inflammation including but not limited to conditions such as
asthma and ARDS as well as other diseases manifested by dysfunctional leukocytes.
Excess production of oxidants is responsible for the activation of MAPK pathways. Excess
production of oxidants is also common to the major atherosclerotic risk factors as well as to
ischaemic reperfusion injury. Lipid peroxidation in cell membranes, cytoplasmic free
10 radicals, together with 'oxidative stress' lead to activation of AP-1 and NF-kB. The JNK
pathway has been established as well as activation of Ras appears to be involved in the
responses. Stimulation of macrophages and endothelial cells by LPS or TNF/IL-1 results in
the activation of MAPK, JNK and P38 pathways. The selective P38 inhibitor SB203580, for
example, has been clearly shown to inhibit production of TNF and IL-1 by LPS- stimulated
15 macrophages as well as TNF, IL-6 and IL-8 by TNF-stimulated HUVECs. Philip Cohen,
*Dissection of Protein Kinase Cascades That Mediate Cellular Response to Cytokines and
Cellular Stress*, in: *Intracellular Signal Transduction, Advances in Pharmacology*, Vol. 36,
Academic Press (1996). MAPK pathways are also activated by shear stress. Atherosclerotic
lesions develop and progress in areas of low and unstable shear stress and not in areas
20 exposed to steady shear. Acute changes in shear stress activate MAPK pathways and chronic
stress desensitizes MAPK and NF-kB pathways indicating that these pathways are activated.
Transient shear stress can activate inflammatory genes partly through activation of the JNK
pathway and AP-1 although the ERK pathway is also activated. Despite major efforts to
develop new therapeutic approaches Adult Respiratory Distress Syndrome (ARDS) (acute
25 pulmonary inflammation characterized by the massive generation of Reactive Oxygen Species
(ROS) within the lung) remains lethal for about 50% of affected patients. Polla, B.S., *et al.*,
Stress Proteins in Inflammation, in: *Stress Inducible Cellular Responses*, Feige, U., *et al.*
Eds., Birkhauser Verlag (1996). Accordingly, the novel human serine/threonine stress-
activated kinase signal transduction molecule described herein (SEQ ID NO:3), and nucleic
30 acid sequences coding therefor, e.g., SEQ ID NO:1 and SEQ ID NO:2, have significant
potential as specific targets that can be exploited diagnostically and therapeutically for the

control of pathophysiologicals relating to inflammation, dysfunctional macrophages, dysfunctional endothelial cells, and related inflammatory diseases including but not limited to conditions such as atherosclerosis, asthma, allergic response, ARDS, heart failure, Atheroma and related disorders.

5 Differentiation, proliferation, growth arrest, or apoptosis of cells depends on the presence of appropriate cytokines and their receptors, as well as the corresponding cellular signal transduction cascades. It remains clear that stress kinase pathways, make critical contributions to transformation. In view of the positioning of raf-MEK1-Erk1/2 downstream of ras, the antiproliferative biological effect of inhibiting signal transduction in the MAP
10 kinase pathway have significant therapeutic potential, applicable, for example, but not limited to tumours harbouring oncogenic ras. Moreover, activation of stress pathway kinases has been implicated in mediating apoptosis by agents such as TNF in model systems. Evidence regarding the involvement of intracellular signalling pathways in the maintenance of cellular survival suggests that survival and mitogenic signals can be separable and that the balance
15 between these signals plays a key role in determining the fate of transformed cells. There are also indications that perturbation of this balance provides selective apoptosis. It would be critical to achieve optimum selectivity between pathways to achieve apoptosis promotion in tumour cells.

Unfortunately, in spite of the introduction of numerous new drugs during the last three
20 decades, only very modest progress can be registered with regard to both cure or survival rates of cancer patients treated with anti-tumor agents. Thus, there is a need for new, more efficient and less toxic compounds. The majority of presently used anti-tumor agents interfere with the biosynthesis of nucleic acids or their intracellular function. Compounds which inhibit uncontrolled growth by interfering with mitogenic signal transduction may act as cytostatic
25 rather than cytotoxic drugs. Furthermore, attenuation of cellular proliferation has frequently been shown to cause tissue differentiation. Finally, blockade of mitogenic stimulation in a cell can result in the induction of apoptosis or programmed cell death. Accordingly, the novel human serine/threonine stress-activated kinase signal transduction molecule described herein (SEQ ID NO:3), and nucleic acid sequences coding therefor, e.g. SEQ ID NO:1 and SEQ ID
30 NO:2, have significant potential as a specific targets that can be exploited diagnostically and therapeutically to control cancer and the proliferation of tumor cells.

Activation of members of each of the MAPK pathways has been demonstrated in response to endothelin, serum, PDGF and TGF β in various types of 'fibroblast'. Dominant negatives of ERK 1 and Rac have been demonstrated, for example, to inhibit expression of a collagen promotor/reporter gene in TGF β -stimulated 3T3 fibroblasts. Moreover, in stellate
5 liver cells, for instance, evidence has been shown that Raf and the JNK pathway interact to control cell proliferation and collagen expression. A number of cytokines, some of which are known to activate SAPKs in cells, have been implicated in cachexia. Accordingly, the novel human serine/threonine stress-activated kinase signal transduction molecule described herein (SEQ ID NO:3), and nucleic acid sequences coding therefor, e.g. SEQ ID NO:1 and SEQ ID
10 NO:2, have significant potential as specific targets that can be exploited diagnostically and therapeutically in the control of dysfunctional fibroblasts, including but not limited to conditions such as pathological fibrosis, and cachexia as well as other diseases manifested by dysfunctional fibroblasts.

The establishment and remodeling of blood vessels is controlled by paracrine signals,
15 many of which are protein ligands that bind and modulate the activity of transmembrane receptor tyrosine kinases (RTKs). The basic view of RTK signaling has come from studies (performed largely in fibroblasts) of ligand-dependent autophosphorylation and activation of the branched Ras pathways. The results suggest that most RTKs are similarly coupled into the intracellular signal transduction cascade and are capable of inducing cell proliferation.
20 Hanahan, D., *Signaling Vascular Morphogenesis and Maintenance*, Science, 227:48 (1997). Angiogenic response of vascular endothelium, endothelial cell proliferation, is one of the first steps in the angiogenic process which has clearly been demonstrated to be induced by hypoxia stress. VEGF, bFGF, and EGF all have been demonstrated to upregulate MAPK in HUVEC cells. Accordingly, the novel human serine/threonine stress-activated kinase signal
25 transduction molecule described herein (SEQ ID NO:3), and nucleic acid sequences coding therefor, e.g. SEQ ID NO:1 and SEQ ID NO:2, have significant potential as specific targets that can be exploited diagnostically and therapeutically to control angiogenesis as well as other manifestations of dysfunctional fibroblasts.

Stress-activated protein kinase (SAPK), members of the ERK family, are activated *in*
30 *situ* by inflammatory stimuli, including tumour-necrosis factor (TNF). TNF is believed to be responsible for the development of insulin resistance associated with obesity. Exposure of

cultured cells to TNF α induces insulin resistance which is believed to be mediated by the Type I TNF α receptor and intracellular signalling mechanisms. In view of the evidence that TNF α is intimately associated with the activation of Stress Activated Protein Kinases (SAPKs), the novel human serine/threonine stress-activated kinase signal transduction molecule described herein (SEQ ID NO:3), and nucleic acid sequences coding therefor, e.g. SEQ ID NO:1 and SEQ ID NO:2, have significant potential as specific targets that can be exploited diagnostically and therapeutically to control Diabetes and related disorders.

The leptin receptor belongs to the cytokine receptor superfamily of which several members have been shown to feed into SAPK pathways. Intracellular signalling pathways utilised by leptin and the potential for regulation of the leptin receptor through "cross-talk" with other signalling pathways is expected to lead to the design of novel therapeutic approaches for the treatment of obesity. Accordingly, the novel human serine/threonine stress-activated kinase signal transduction molecule described herein (SEQ ID NO:3), and nucleic acid sequences coding therefor, e.g. SEQ ID NO:1 and SEQ ID NO:2, have significant potential as specific targets that can be exploited diagnostically and therapeutically to control Obesity and related disorders.

Compounds which modulate or inactivate specific signal transduction molecules integral to specific cytosolic pathways generally have significant potential for the ability to modulate or attenuate downstream physiological responses. Accordingly, the ability to screen for compounds which modulate the activity of the native human stress-activated serine/threonine signal transduction kinase molecule described herein is of paramount importance toward the development of therapeutic agents.

Functional constructions

The cDNA for the kinase of the present invention has been subcloned into several expression vectors as examples of providing recombinant protein useful for drug screen assays and/or for employment *in vivo* or otherwise of the physiological role of the kinase. Expression studies are performed using either constitutive, induced, or tissue specific expression systems in mammalian cell lines. The constructs herein described are example embodiments to be used to transfect mammalian cell lines, e.g., U937 and other well-known cell lines, in order to provide recombinant protein suitable for use in drug screen assays. Constructs are contemplated for over-expressing the wild-type and/or dominant negative

-33-

kinase of the present invention for characterization its physiological role including activation stimuli, identification of signal transduction pathways, identification of protein-protein interactions, as well as validation of optimal drug candidates.

The nucleic acid sequences which encode the novel kinase can be subcloned into an expression vector in the antisense orientation to provide a tool for producing gene knock-out studies of gene function. Down-regulation of the native signal transduction kinase molecule described herein is also contemplated using antisense expression.

FIG.7 displays SEQ ID NO:6 and SEQ ID NO:7 which are oligonucleotide PCR primers used to produce full-length nucleic acid sequence amplicon pertaining to the novel human stress activated kinase. A shuttle construct containing a nucleic acid coding region of the novel signal transduction kinase was produced by "TA" cloning of the PCR amplicon (SEQ ID NO:8), described *infra*, into the pCR2.1 vector (Invitrogen, Carlsbad, CA).

A GST-Kinase fusion expression vector, designed to express a N-terminal Glutathione S-transferase fusion protein (produced using pGEX5x1, Pharmacia Biotech, Inc., Piscataway, New Jersey), was constructed by subcloning the signal transduction kinase cDNA from the pCR2.1 vector into the pGEX5x1 vector. The signal transduction kinase cDNA was excised from the pCR2.1 vector by restriction digestion using EcoRI and EcoICRI. The fragment was gel purified, then inserted into pGEX5x1 which had been digested with EcoRI and SmaI restriction endonucleases and gel purified. Insertion of the signal transduction kinase cDNA into pGEX5x1 yielded a chimeric EcoICRI:SmaI site which was no longer recognizable by either restriction enzyme. The ligated DNA was used to transform DH5 α competent *E.coli* cells (Life Technologies, Gaithersburg, MD). Single, isolated transformed *E.coli* colonies were grown in selective media (LB broth, carbenicillin) overnight at 37°C and subsequently used to prepare plasmid DNA (Qiagen Plasmid DNA preparation kit). Clones were sequenced (ABI PRISM™ Dye Terminator Cycle sequencing on ABI PRISM™377 automated sequencer) to identify correctly inserted cDNA. A correctly cloned construct was selected and used to transform BL21 competent *E. coli* cells (Novagen, Inc.) for expression. Expression and purification of the GST/human kinase fusion protein is described *infra* in EXAMPLE IX.

30

Kinase activity

A kinase activity assay was used to demonstrate the novel signal transduction kinase SEQ ID NO:3 is a functional kinase. Recombinant GST-TEN1 was expressed in BL21 (DE3) *E.coli* cells and purified as outlined in Example IX. The recombinant kinase was tested for catalytic activity as shown in Example II. See FIG.11. The lane containing GST in place of GST-TEN1 kinase shows no phosphorylation and indicates that phosphorylation in the presence of TEN1 is due to its kinase activity and not due to a co-purified contaminant.

To construct a His₆-Xpress epitope tagged novel human signal transduction kinase mammalian expression vector the novel kinase protein coding region was excised from the pCR2.1 construct using EcoRI (5') and EcoICRI (3') and inserted into the pcDNA3.1HisC vector (Invitrogen) at the EcoRI (5') and EcoRV (3') sites. Joining of the EcoICRI site from the pCR2.1 construct to the EcoRV site from the pcDNA3.1HisC vector results in the loss of both sites due to a blunt ended ligation. The resulting construct encodes an epitope tagged kinase protein containing 6 histidine residues and the "Xpress" antibody epitope at the N-terminus of the novel kinase protein. Expression of the epitope tagged novel signal transduction kinase in eukaryotic cells is effected by transfecting mammalian cell lines either transiently or stably with expression vectors containing the novel kinase coding region. See, e.g., EXAMPLES X and XI. The transformed cells serve as sources of kinase for screening candidate compound modulators, *inter alia*.

Generally acceptable vectors

In accordance with the present invention, polynucleotide sequences which encode the novel kinase, fragments of the polypeptide, fusion proteins or functional equivalents thereof may be used in recombinant DNA molecules that direct the expression of the signal transduction molecule in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence, may be used to clone and express the novel human signal-transduction kinase. As will be understood by those of skill in the art, it may be advantageous to produce novel kinase-encoding nucleotide sequences possessing non-naturally occurring codons.

Specific initiation signals may also be required for efficient translation of a signal-transduction kinase sequence. These signals include the ATG initiation codon and adjacent sequences. In cases where the novel kinase, its initiation codon and upstream sequences are

inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic.

Cloned signal transduction kinase cDNA obtained through the methods described herein may be recombinantly expressed by molecular cloning into an expression vector containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce the kinase polypeptide. Techniques for such manipulations are fully described in Sambrook, J., *et al.*, Molecular Cloning Second Edition, Cold Spring Harbor Press (1990), and are well known in the art.

Expression vectors are described herein as DNA sequences for the transcription of cloned copies of genes and the translation of their mRNAs in an appropriate host cell. Such vectors can be used to express nucleic acid sequences in a variety of hosts such as bacteria, bluegreen algae, plant cells, insect cells, fungal cells, human, and animal cells. Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast, or bacteria-animal cells, or bacteria-fungal cells, or bacteria-invertebrate cells.

A variety of mammalian expression vectors may be used to express the recombinant human kinase molecule disclosed herein in mammalian cells. Commercially available mammalian expression vectors which are suitable for recombinant expression, include but are not limited to, pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and IZD35 (ATCC 37565), pLXIN and pSIR (CLONTECH), pIRES-EGFP (CLONTECH). INVITROGEN corporation provides a wide variety of commercially available mammalian expression vector/systems which can be effectively used with the present invention. INVITROGEN, Carlsbad, CA. *See, also*, PHARMINGEN products, vectors and systems, San Diego, CA.

Baculoviral expression systems may also be used with the present invention to produce high yields of biologically active protein. Vectors such as the CLONETECH,

BacPak™ Baculovirus expression system and protocols are preferred which are commercially available. CLONTECH, Palo Alto, CA. Miller, L.K., *et al.*, Curr. Op. Genet. Dev. 3:97 (1993); O'Reilly, D.R., *et al.*, *Baculovirus Expression Vectors: A Laboratory Manual*, 127. Vectors such as the INVITROGEN, MaxBac™ Baculovirus expression system, insect cells, and protocols are also preferred which are commercially available. INVITROGEN, Carlsbad, CA.

Example host cells

Host cells transformed with a nucleotide sequence which encodes the human kinase of the present invention may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. Particularly preferred embodiments of the present invention are host cells transformed with a purified polynucleotide comprising a nucleic acid sequence encoding the polypeptide having the sequence substantially as depicted in SEQ ID NO:3 or a biologically active fragment thereof. Cells of this type or preparations made from them may be used to screen for pharmacologically active modulators of the novel human signal-transduction kinase activity.

Eukaryotic recombinant host cells are especially preferred. Examples include but are not limited to yeast, mammalian cells including but not limited to cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which may be suitable and which are commercially available, include but are not limited to, L cells L-M(TK-) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The expression vector may be introduced into host cells expressing the novel kinase via any one of a number of techniques including but not limited to transformation, transfection, lipofection, protoplast fusion, and electroporation. Commercially available kits applicable for use with the present invention for heterologous expression, including well-characterized vectors, transfection reagents and conditions, and cell culture materials are well-established and readily available. CLONTECH, Palo Alto, CA; INVITROGEN, Carlsbad,

-37-

CA: PHARMINGEN, San Diego, CA; STRATAGENE, LaJolla, CA. The expression vector-containing cells are clonally propagated and individually analyzed to determine the level of novel kinase protein production. Identification of host cell clones which express the novel kinase may be performed by several means, including but not limited to immunological
5 reactivity with antibodies described herein, and/or the presence of host cell-associated specific kinase activity, and/or the ability to covalently cross-link specific substrate to the novel kinase with the bifunctional cross-linking reagent disuccinimidyl suberate or similar cross-linking reagents.

The signal transduction molecule of the present invention may also be expressed as a
10 recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals (Porath, J., Protein Exp. Purif. 3:263 (1992)), protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS
15 extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor XA or enterokinase (Invitrogen, San Diego CA) between the purification domain and TMP is useful to facilitate purification.

Systems such as the CLONTECH, TALON™ nondenaturing protein purification kit for purifying 6xHis-tagged proteins under native conditions and protocols are preferred which
20 are commercially available. CLONTECH, Palo Alto, CA.

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which
25 cleaves a nascent form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, NIH-3T3, HEK293 etc., have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

30 For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the novel kinase may be transformed

-38-

using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its
5 presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

The human kinase can be produced in the yeast *S.cerevisiae* following the insertion of the optimal cDNA cistron into expression vectors designed to direct the intracellular or
10 extracellular expression of the heterologous protein. In the case of intracellular expression, vectors such as EmBLyex4 or the like are ligated to the beta subunit cistron. See, e.g., Rinas, U., *et al.*, *Biotechnology*, 8:543 (1990); Horowitz, B., *et al.*, *J. Biol. Chem.*, 265:4189 (1989). For extracellular expression, the kinase cistron is ligated into yeast expression vectors which may employ any of a series of well-characterized secretion signals. The levels of expressed
15 novel kinase are determined by the assays described herein.

A variety of protocols for detecting and measuring the expression of the novel kinase, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay
20 utilizing monoclonal antibodies reactive to two non-interfering epitopes may be employed. Well known competitive binding techniques may also be employed. See, e.g., Hampton, R., *et al.* (1990), *Serological Methods - a Laboratory Manual*, APS Press. St Paul Minn.; Maddox, D.E., *et al.*, *J. Exp. Med.* 158:1211.

Screening assays

25 The present invention is also directed to methods for screening for compounds which modulate the expression of DNA or RNA encoding the novel kinase polypeptide, as well as the function of the signal-transduction kinase polypeptide *in vivo*. Compounds which modulate these activities may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules. Compounds may modulate by increasing or attenuating the expression of DNA or
30 RNA encoding the signal-transduction kinase polypeptide, or the function thereof.

Compounds that modulate the expression of DNA or RNA encoding the signal-transduction

kinase polypeptide or the function of the polypeptide may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample.

5 The signal-transduction kinase described herein, its immunogenic fragments or oligopeptides can be used for screening therapeutic compounds in any of a variety of drug screening techniques. The fragment employed in such a test may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The abolition of activity or the formation of binding complexes, between the signal-transduction kinase polypeptide
10 and the agent being tested, may be measured. Accordingly, the present invention provides a method for screening a plurality of compounds for specific binding affinity with the signal-transduction kinase polypeptide or a fragment thereof, comprising providing a plurality of compounds; combining the signal-transduction kinase polypeptide of the present invention or a fragment thereof with each of a plurality of compounds for a time sufficient to allow binding
15 under suitable conditions; and detecting binding of the kinase polypeptide, or fragment thereof, to each of the plurality of compounds, thereby identifying the compounds which specifically bind the signal-transduction kinase polypeptide.

Methods of identifying compounds that modulate the activity of a signal-transduction kinase polypeptide are generally preferred, which comprise combining a candidate compound
20 modulator of a signal-transduction kinase activity with a polypeptide of a signal-transduction kinase having the sequence substantially as depicted in SEQ ID NO:3, and measuring an effect of the candidate compound modulator on the kinase activity.

Methods of identifying compounds that modulate the activity of a signal-transduction kinase, are also preferred which comprise combining a candidate compound modulator of a
25 signal-transduction kinase activity with a host-cell expressing the polypeptide of a signal-transduction kinase molecule having the sequence substantially as depicted in SEQ ID NO:3, and measuring an effect of the candidate compound modulator on the kinase activity.

Preferred cellular assays for inhibitors of the kinase fall into two general categories: 1) direct measurement of the kinase activity, and 2) measurement of downstream events in the
30 signaling cascade. These methods can employ the endogenous kinase, or the overexpressed recombinant kinase.

Methods of identifying compounds that modulate the biological activity of a signal-transduction kinase are generally preferred, which comprise combining a candidate compound modulator of a signal-transduction kinase activity with a polypeptide of a signal-transduction kinase having the sequence substantially as depicted in SEQ ID NO:3, and measuring an effect of the candidate compound modulator on the biological activity. Methods of identifying compounds that modulate the pharmacological activity of a signal-transduction kinase are also preferred, which comprise combining a candidate compound modulator of a signal-transduction kinase activity with a polypeptide of a signal-transduction kinase having the sequence substantially as depicted in SEQ ID NO:3, and measuring an effect of the candidate compound modulator on the pharmacological activity.

Methods of identifying compounds that modulate the activity of a signal-transduction kinase, are particularly preferred which comprise combining a candidate compound modulator of a signal-transduction kinase activity with a host-cell expressing the polypeptide of a signal-transduction kinase molecule having the sequence substantially as depicted in SEQ ID NO:3, and measuring an effect of the candidate compound modulator on the biological activity activity. Methods of identifying compounds that modulate the activity of a signal-transduction kinase, are also particularly preferred which comprise combining a candidate compound modulator of a signal-transduction kinase activity with a host-cell expressing the polypeptide of a signal-transduction kinase molecule having the sequence substantially as depicted in SEQ ID NO:3, and measuring an effect of the candidate compound modulator on the pharmacological activity.

Compounds which are identified generally according to methods described, contemplated, and referenced herein that modulate the biological and/or pharmacological activity of a signal-transduction molecule of the sequence substantially as depicted in SEQ ID NO:3 are especially preferred embodiments of the present invention.

An especially preferred embodiment of the present invention is a method for treatment of a patient in need of such treatment for a condition which is mediated by the human signal-transduction molecule described herein, e.g., SEQ ID NO:3, comprising administration of a therapeutically effective amount of a human signal-transduction modulating compound identified using sequences substantially as depicted in SEQ ID NO:2 and/or SEQ ID NO:3 as a pharmacological target in methods contemplated herein.

-41-

In order to measure the cellular activity of the kinase, the source may be a whole cell lysate, prepared by one to three freeze-thaw cycles in the presence of standard protease inhibitors. Alternatively, the kinase may be partially or completely purified by standard protein purification methods. Finally, the kinase may be purified by affinity chromatography using specific antibody for the C terminal regulatory domain described herein or by ligands specific for the epitope tag engineered into the recombinant kinase moreover described herein. The kinase preparation may then be assayed for activity as described, for example, in EXAMPLE II.

A filter assay based on the protocol of Reuter *et al.* (1995) is also used to screen for compounds which modulate the activity of the novel kinase described herein: Starting with MBP coated 96-well FlashPlates® (NEN™ Life Science Products) reaction buffer (3X kinase reaction buffer (KRB) contains: 60 mM HEPES (pH 7.5), 30 mM magnesium acetate, 0.15 mM ATP, 3 mM DTT, 0.03 mM sodium orthovanadate) is added, 0.25µCi [γ ³³P]-ATP at a concentration no greater than 1 µg/ml, (determined by titration of individual enzyme preparations for a concentration that allows kinetic determinations over a 1 hour time course of the human kinase) of the human kinase are added to each well and incubated 1 hour at 30°C in the presence or absence of 10µM test compound. Total reaction volume is 100µL. The reaction is stopped by the addition of EDTA (pH 7.0) to a final concentration of 80mM. The samples are centrifuged and 50µL of the supernatant spotted on p81 cation-exchange filter paper (Whatman, No. 3698 915). The filters are then washed 3 times in 200mL of 180mM H₃PO₄ (5-10min each), and once in 200mL of 96% ethanol. After air drying the filters, radioactivity is determined by Cerenkov counting in a scintillation counter. Compounds which inhibit kinase activity ≥50 percent at 10µM are indicated by a >50% reduction in scintillation counts. Specificity and selectivity studies is determined by titration of inhibitory compounds to determine the IC₅₀ (or other standard quantitation well known in the art for comparison) and by the substitution of other kinases in the assay. For example, determination of relative inhibitory activity of the kinase in comparison to recombinant SOK-1 and/or mst-3, expressed and isolated in a similar manner, assayed under similar conditions, provides selectivity data. Reuter, C.W.M., Catling, A.D. and Weber, M.J., *Immune Complex Kinase Assays for Mitogen-Activated Protein Kinase and MEK*, Methods In Enzymology, 255:245 (1995).

See EXAMPLES VI and VII.

To evaluate the ability of a candidate agent to inhibit human tumor growth, human tumor cells are injected into SCID mice (severe combined immunodeficiency) to form palpable tumor masses. The effects of an candidate agent in inhibiting tumor growth can be determined as follows. Approximately 1×10^7 cells of the CCL 221 cell line (ATCC, Rockville, Md.), a human ras-dependent colon adenocarcinoma cell line, is suspended in 100 μ l DMEM and injected subcutaneously into SCID mice, such that two tumors per mouse are formed. SCID mice receive CCL 221 cells and the tumors are grown for 7 days without treatment; on the 7th day (Day 0) tumor maximal diameters and animal weights are recorded and the mean tumor size for the mice is determined. On Day 1 (eight days following tumor cell injection), treatment of the mice with candidate agent or vehicle alone is begun. One group of the mice (controls) are injected intraperitoneally with 0.2 ml of vehicle and a second group of mice received agent by intraperitoneal injection. Various doses of agent can be tested in separate groups of mice. On Day 7 and Day 14, animal weight and maximal tumor diameter is measured. Average maximal tumor size for each group on Day 0, Day 7, and Day 14 are compared. Day 14, one high dose animal was followed for an additional to determine whether the agent produces a dose-dependent inhibition of tumor growth. Toxicity effects can be examined by tracking mice weight and by harvesting lungs, livers, and spleens of the animals for histological staining.

Compounds which are identified generally according to methods described and referenced herein that modulate the activity of a human signal-transduction kinase comprised of the sequence substantially as depicted in SEQ ID NO:3 are especially preferred embodiments of the present invention.

An especially preferred embodiment of the present invention is a method for treatment of a patient in need of such treatment for a condition which is mediated by the human signal-transduction kinase described herein comprising administration of a therapeutically effective amount of a human signal-transduction kinase modulating compound.

Yeast 2-hybrid system

In another embodiment of the invention, a nucleic acid sequence which encodes a human signal-transduction kinase molecule substantially as depicted in SEQ ID NO:3 or a biologically active fragment thereof may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening compounds for modulation of biological activity, it may be useful to encode a chimeric kinase molecule as described herein for expression in heterologous host cells. Chimeric constructs may also be used to express a 'bait', according to methods well known using a yeast two-hybrid system, to identify accessory native peptides that may be associated with the novel human signal-transduction kinase molecule described herein. Fields, S., *et al.*, Trends Genet., 10:286 (1994); Allen, J.B., *et al.*, TIBS, 20:511 (1995). A yeast two-hybrid system has been described wherein protein:protein interactions can be detected using a yeast-based genetic assay via reconstitution of transcriptional activators. Fields, S., Song, O., Nature 340:245 (1989). The two-hybrid system used the ability of a pair of interacting proteins to bring a transcription activation domain into close proximity with a DNA-binding site that regulates the expression of an adjacent reporter gene. Commercially available systems such as the CLONTECH, Matchmaker™ systems and protocols may be used with the present invention. CLONTECH, Palo Alto, CA. See also, Mendelsohn, A.R., Brent, R., Curr. Op. Biotech., 5:482 (1994); Phizicky, E.M., Fields, S., Microbiological Rev., 59(1):94 (1995); Yang, M., *et al.*, Nucleic Acids Res., 23(7):1152 (1995); Fields, S., Sternglanz, R., TIG, 10(8):286 (1994); and US Patents 5,283,173, *System to Detect Protein-Protein Interactions*, and 5,468,614, which are incorporated herein by reference.

Antibodies

Monospecific antibodies to the signal transduction kinase polypeptide of the present invention are purified from mammalian antisera containing antibodies reactive against the polypeptide or are prepared as monoclonal antibodies reactive with signal transduction kinase polypeptide using the technique of Kohler and Milstein, Nature, 256:495 (1975). Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for the novel signal transduction kinase. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope, such as those associated with the novel signal transduction kinase.

as described. Novel signal transduction kinase specific antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with rabbits being preferred, with an appropriate concentration of the human signal transduction kinase either with or without an immune adjuvant.

5 Preimmune serum is collected prior to the first immunization. Each animal receives between about 0.1 mg and about 1000 mg of signal transduction kinase polypeptide associated with an acceptable immune adjuvant. Such acceptable adjuvants include, but are not limited to, Freund's complete, Freund's incomplete, alum-precipitate, water in oil emulsion containing *Corynebacterium parvum* and tRNA. The initial immunization consists of signal transduction
10 kinase polypeptide in, preferably, Freund's complete adjuvant at multiple sites either subcutaneously (SC), intraperitoneally (IP) or both. Each animal is bled at regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the initial immunization. Those animals receiving booster injections are generally given an equal amount of the antigen in Freund's incomplete adjuvant by the same
15 route. Booster injections are given at about three week intervals until maximal titers are obtained. At about 7 days after each booster immunization or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots are stored at about -20°C.

Monoclonal antibodies (mAb) reactive with the signal transduction kinase polypeptide
20 are prepared by immunizing inbred mice, preferably Balb/c, with the signal transduction kinase polypeptide. The mice are immunized by the IP or SC route with about 0.1 mg to about 10 mg, preferably about 1 mg, of signal transduction kinase polypeptide in about 0.5 ml buffer or saline incorporated in an equal volume of an acceptable adjuvant, as discussed above. Freund's complete adjuvant is preferred. The mice receive an initial immunization on day 0
25 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of about 0.1 to about 10 mg of signal transduction kinase polypeptide in a buffer solution such as phosphate buffered saline by the intravenous (IV) route. Lymphocytes, from antibody positive mice, preferably splenic lymphocytes, are obtained by removing spleens from immunized mice by standard procedures known in the art. Hybridoma
30 cells are produced by mixing the splenic lymphocytes with an appropriate fusion partner, preferably myeloma cells, under conditions which will allow the formation of stable

hybridomas. Fusion partners may include, but are not limited to: mouse myelomas P3/NS1/Ag 4-1; MPC-11; S-194 and Sp 2/0, with Sp 2/0 being preferred. The antibody producing cells and myeloma cells are fused in polyethylene glycol, about 1000 molecular weight, at concentrations from about 30% to about 50%. Fused hybridoma cells are selected
5 by growth in hypoxanthine, thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected from growth positive wells on about days 14, 18, and 21 and are screened for antibody production by an immunoassay such as solid phase immunoradioassay (SPIRA) using the human signal transduction kinase polypeptide as the antigen. The culture fluids are also tested
10 in the Ouchterlony precipitation assay to determine the isotype of the mAb. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, Soft Agar Techniques, in Tissue Culture Methods and Applications, Kruse and Paterson, Eds., Academic Press, 1973.

Monoclonal antibodies are produced *in vivo* by injection of pristane primed Balb/c
15 mice, approximately 0.5 ml per mouse, with about 2×10^6 to about 6×10^6 hybridoma cells about 4 days after priming. Ascites fluid is collected at approximately 8-12 days after cell transfer and the monoclonal antibodies are purified by techniques known in the art.

In vitro production of the anti- human kinase polypeptide mAb is carried out by growing the hybridoma in DMEM containing about 2% fetal calf serum to obtain sufficient
20 quantities of the specific mAb. The mAb are purified by techniques known in the art.

Diagnostic assays

Antibody titers of ascites or hybridoma culture fluids are determined by various serological or immunological assays which include, but are not limited to, precipitation, passive agglutination, enzyme-linked immunosorbent antibody (ELISA) technique and
25 radioimmunoassay (RIA) techniques. Similar diagnostic assays are used to detect the presence of the novel signal transduction kinase polypeptide in body fluids or tissue and cell extracts.

Diagnostic assays using the human signal-transduction kinase polypeptide specific antibodies are useful for the diagnosis of conditions, disorders or diseases characterized by abnormal expression of the signal-transduction kinase polypeptide or expression of genes
30 associated with abnormal cell growth. Diagnostic assays for the signal-transduction kinase polypeptide of this invention include methods utilizing the antibody and a label to detect the

human kinase polypeptide in human body fluids, cells, tissues or sections or extracts of such tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule, a myriad of which are well-known to those skilled in the art.

A variety of protocols for measuring the kinase polypeptide, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on the human signal-transduction kinase polypeptide is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, D.E. *et al.*, *J. Exp. Med.* 158:1211 (1983); Sites, D.P., *et al.*, *Basic and Clinical Immunology*, Ch.22, 4th Ed., Lange Medical Publications, Los Altos, CA (1982); U.S. Patents No. 3,654,090, No. 3,850,752; and No. 4,016,043.

In order to provide a basis for the diagnosis of disease, normal or standard values for the human signal-transduction kinase polypeptide expression must be established. This is accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with antibody to the human kinase polypeptide under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by comparing it with a dilution series of positive controls where a known amount of antibody is combined with known concentrations of purified human signal-transduction kinase polypeptide. Then, standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by a disorder or disease related to the human kinase polypeptide expression. Deviation between standard and subject values establishes the presence of the disease state.

Kits containing human stress-activated signal-transduction kinase nucleic acid, antibodies to a kinase polypeptide, or protein may be prepared. Such kits are used to detect heterologous nucleic acid which hybridizes to kinase nucleic acid, or to detect the presence of protein or peptide fragments in a sample. Such characterization is useful for a variety of purposes including, but not limited to, forensic analyses and epidemiological studies.

The DNA molecules, RNA molecules, recombinant protein and antibodies of the present invention may be used to screen and measure levels of the novel kinase DNA, RNA or protein. The recombinant proteins, DNA molecules, RNA molecules and antibodies lend themselves to the formulation of kits suitable for the detection and typing of the human
5 signal-transduction kinase. Such a kit would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant human kinase or anti-kinase antibodies suitable for detecting the novel kinase. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the like.

10 Polynucleotide sequences which encode the novel kinase may be used for the diagnosis of conditions or diseases with which the expression of the novel human stress-activated kinase is associated. For example, polynucleotide sequences encoding the signal-transduction molecule may be used in hybridization or PCR assays of fluids or tissues from biopsies to detect expression of the kinase. The form of such qualitative or quantitative
15 methods may include Southern or northern analysis, dot blot or other membrane-based technologies; PCR technologies; dip stick, pin, chip and ELISA technologies. All of these techniques are well known in the art and are the basis of many commercially available diagnostic kits. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regime in animal studies, in clinical trials, or in monitoring the treatment
20 of an individual patient. Once disease is established, a therapeutic agent is administered and a treatment profile is generated. Such assays may be repeated on a regular basis to evaluate whether the values in the profile progress toward or return to the normal or standard pattern. Successive treatment profiles may be used to show the efficacy of treatment over a period of several days or several months.

25 Polynucleotide sequences which encode the novel kinase may also be employed in analyses to map chromosomal locations, e.g., screening for functional association with disease markers. Moreover the sequences described herein are contemplated for use to identify human sequence polymorphisms and possible association with disease as well as analyses to select optimal sequence from among possible polymorphic sequences for the design of
30 compounds to modulate the biological activity. Furthermore the sequences are contemplated

as screening tools for use in the identification of appropriate human subjects and patients for therapeutic clinical trials.

Purification via affinity columns

It is readily apparent to those skilled in the art that methods for producing antibodies
5 may be utilized to produce antibodies specific for the human kinase polypeptide fragments, or the full-length nascent human kinase polypeptide. Specifically, it is readily apparent to those skilled in the art that antibodies may be generated which are specific for the fully functional receptor or fragments thereof.

Kinase polypeptide antibody affinity columns are made by adding the antibodies to
10 Affigel-10 (Biorad), a gel support which is activated with N hydroxysuccinimide esters such that the antibodies form covalent linkages with the agarose gel bead support. The antibodies are then coupled to the gel via amide bonds with the spacer arm. The remaining activated esters are then quenched with 1M ethanolamine HCl (pH 8). The column is washed with water followed by 0.23M glycine HCl (pH 2.6) to remove any non-conjugated antibody or
15 extraneous protein. The column is then equilibrated in phosphate buffered saline (pH 7.3) with appropriate detergent and the cell culture supernatants or cell extracts containing human signal-transduction kinase polypeptide made using appropriate membrane solubilizing detergents are slowly passed through the column. The column is then washed with phosphate buffered saline/detergent until the optical density falls to background, then the protein is
20 eluted with 0.23M glycine-HCl (pH 2.6)/detergent. The purified human signal-transduction kinase polypeptide is then dialyzed against phosphate buffered saline/detergent.

Recombinant kinase molecules can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for full length nascent human kinase polypeptide, or polypeptide fragments of the kinase molecule.

25 Human kinase polypeptides described herein may be used to affinity purify biological effectors from native biological materials, e.g. disease tissue. Affinity chromatography techniques are well known to those skilled in the art. A human signal-transduction kinase peptide described herein or an effective fragment thereof, is fixed to a solid matrix, e.g. CNBr activated Sepharose according to the protocol of the supplier (Pharmacia, Piscataway, NJ),
30 and a homogenized/buffered cellular solution containing a potential molecule of interest is

passed through the column. After washing, the column retains only the biological effector which is subsequently eluted, e.g., using 0.5M acetic acid or a NaCl gradient.

Antisense molecules

The cDNA sequence SEQ ID NO:1 provided herein, may be used in another embodiment of the invention to study the physiological relevance of the novel human signal-transduction kinase in cells, especially cells of hematopoietic origin, by knocking out the endogenous gene by use of anti-sense constructs.

Oligomers of 12-21 nucleotides are most preferred in the practice of the present invention, particularly oligomers of 12-18 nucleotides. Oligos are, in principle, effective for inhibiting translation of the transcript, and capable of inducing the effects herein described. Translation is most effectively inhibited by blocking the mRNA at a site at or near the initiation codon. Thus, oligonucleotides complementary to the 5' -terminal region of the human kinase mRNA transcript are preferred. Secondary or tertiary structure which might interfere with hybridization is minimal in this region. Moreover, sequences that are too distant in the 3' direction from the initiation site can be less effective in hybridizing the mRNA transcripts because of a "read-through" phenomenon whereby the ribosome appears to unravel the antisense/sense duplex to permit translation of the message. Oligonucleotides which are complementary to and hybridizable with any portion of the novel human signal-transduction kinase mRNA are contemplated for therapeutic use

U.S. Patent No. 5,639,595, *Identification of Novel Drugs and Reagents*, issued Jun. 17, 1997, wherein methods of identifying oligonucleotide sequences that display *in vivo* activity are thoroughly described, is herein incorporated by reference. Expression vectors containing random oligonucleotide sequences derived from previously known polynucleotides are transformed/transfected into cells. The cells are then assayed for a phenotype resulting from the desired activity of the oligonucleotide. Once cells with the desired phenotype have been identified, the sequence of the oligonucleotide having the desired activity can be identified. Identification may be accomplished by recovering the vector or by polymerase chain reaction (PCR) amplification and sequencing the region containing the inserted nucleic acid material.

Nucleotide sequences that are complementary to the novel signal-transduction kinase polypeptide encoding polynucleotide sequence can be synthesized for antisense therapy.

These antisense molecules may be DNA, stable derivatives of DNA such as

-50-

phosphorothioates or methylphosphonates, RNA, stable derivatives of RNA such as 2'-O-alkylRNA, or other oligonucleotide mimetics. U.S. Patent No. 5,652,355, *Hybrid Oligonucleotide Phosphorothioates*, issued July 29, 1997, and U.S. Patent No. 5,652,356, *Inverted Chimeric and Hybrid Oligonucleotides*, issued July 29, 1997, which describe the synthesis and effect of physiologically-stable antisense molecules, are incorporated by reference. Signal-transduction kinase antisense molecules may be introduced into cells by microinjection, liposome encapsulation or by expression from vectors harboring the antisense sequence. Antisense therapy may be particularly useful for the treatment of diseases where it is beneficial to reduce the signal-transduction kinase activity.

10 Gene therapy

A human signal-transduction kinase polypeptide described herein may administered to a subject *via* gene therapy. Moreover, a polypeptide of the present invention may be delivered to the cells of target organs in this manner. Conversely, signal-transduction kinase polypeptide *antisense* gene therapy may be used to reduce the expression of the polypeptide in the cells of target organs. The human signal-transduction kinase polypeptide coding region can be ligated into viral vectors which mediate transfer of the kinase polypeptide DNA by infection of recipient host cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, polio virus and the like. *See, e.g.*, U.S. Patent No. 5,624,820, *Episomal Expression Vector for Human Gene Therapy*, issued April 29, 1997. Nucleic acid coding regions of the present invention are incorporated into effective eukaryotic expression vectors, which are directly administered or introduced into somatic cells for gene therapy (a nucleic acid fragment comprising a coding region, preferably mRNA transcripts, may also be administered directly or introduced into somatic cells). *See, e.g.*, U.S. Patent No. 5,589,466, issued Dec. 31, 1996. Such nucleic acids and vectors may remain episomal or may be incorporated into the host chromosomal DNA as a provirus or portion thereof that includes the gene fusion and appropriate eukaryotic transcription and translation signals, i.e., an effectively positioned RNA polymerase promoter 5' to the transcriptional start site and ATG translation initiation codon of the gene fusion as well as termination codon(s) and transcript polyadenylation signals effectively positioned 3' to the coding region. Alternatively, the human signal-transduction kinase polypeptide DNA can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted DNA transfer using

ligand-DNA conjugates or adenovirus-ligand-DNA conjugates, lipofection membrane fusion or direct microinjection. These procedures and variations thereof are suitable for *ex vivo*, as well as *in vivo* human signal-transduction kinase polypeptide gene therapy according to established methods in this art.

5 Per diagnostics

The nucleic acid sequence, oligonucleotides, fragments, portions or antisense molecules thereof, may be used in diagnostic assays of body fluids or biopsied tissues to detect the expression level of the novel human signal-transduction kinase molecule. For example, sequences designed from the cDNA sequence SEQ ID NO:1 or sequences
10 comprised in SEQ ID NO:2 can be used to detect the presence of the mRNA transcripts in a patient or to monitor the modulation of transcripts during treatment.

One method for amplification of target nucleic acids, or for later analysis by hybridization assays, is known as the polymerase chain reaction ("PCR") or PCR technique. The PCR technique can be applied to detect sequences of the invention in suspected samples
15 using oligonucleotide primers spaced apart from each other and based on the genetic sequence, e.g., SEQ ID NO:1, set forth herein. The primers are complementary to opposite strands of a double stranded DNA molecule and are typically separated by from about 50 to 450 nucleotides or more (usually not more than 2000 nucleotides). This method entails preparing the specific oligonucleotide primers followed by repeated cycles of target DNA
20 denaturation, primer binding, and extension with a DNA polymerase to obtain DNA fragments of the expected length based on the primer spacing. One example embodiment of the present invention is a diagnostic composition for the identification of a polynucleotide sequence comprising the sequence substantially as depicted in SEQ ID NO:2 comprising the PCR primers substantially as depicted in SEQ ID NO:6 and SEQ ID NO:7. The degree of
25 amplification of a target sequence is controlled by the number of cycles that are performed and is theoretically calculated by the simple formula 2^n where n is the number of cycles. See, e.g., Perkin Elmer, *PCR Bibliography*, Roche Molecular Systems, Branchburg, New Jersey; CLONTECH products, Palo Alto, CA: U.S. Patent No. 5,629,158, *Solid Phase Diagnosis of Medical Conditions*, issued May 13, 1997. See also, EXAMPLE VIII.

Compositions

Pharmaceutically useful compositions comprising the novel human kinase polypeptide DNA, human kinase polypeptide RNA, antisense sequences, or the human kinase polypeptide, or variants and analogs which have the human kinase activity or otherwise modulate its activity, may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in *Remington's Pharmaceutical Sciences* (Maack Publishing Co, Easton, PA). To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the protein, DNA, RNA, or modulator.

Therapeutic or diagnostic compositions of the invention are administered to an individual in amounts sufficient to treat or diagnose human signal-transduction kinase polypeptide related disorders. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration.

The term "chemical derivative" describes a molecule that contains additional chemical moieties which are not normally a part of the base molecule. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are described in a variety of texts, such as *Remington's Pharmaceutical Sciences*.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. The therapeutically effective dose can be estimated initially either in cell culture assays, eg, of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. A therapeutically effective dose refers to that amount of protein or its antibodies, antagonists, or inhibitors which ameliorate the symptoms or condition. The exact dosage is chosen by the individual physician in view of the patient to be treated.

Compounds identified according to the methods disclosed herein may be used alone at appropriate dosages defined by routine testing in order to obtain optimal modulation of a signal-transduction kinase, or its activity while minimizing any potential toxicity. In addition, co-administration or sequential administration of other agents may be desirable.

5 The pharmaceutical compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, oral and intramuscular. Administration of pharmaceutical compositions is accomplished orally or parenterally. Methods of parenteral delivery include topical, intra-arterial (directly to the tissue), intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal
10 administration. The present invention also has the objective of providing suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the novel methods of treatment of the present invention. The compositions containing compounds identified according to this invention as the active ingredient for use in the modulation of signal-transduction kinase can be administered in a wide variety of therapeutic dosage forms in
15 conventional vehicles for administration. For example, the compounds can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or
20 intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. An effective but non-toxic amount of the compound desired can be employed as a signal-transduction kinase modulating agent.

The daily dosage of the products may be varied over a wide range from 0.01 to 1.000 mg per adult human/per day. For oral administration, the compositions are preferably
25 provided in the form of scored or unscored tablets containing 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, and 50.0 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. An effective amount of the drug is ordinarily supplied at a dosage level of from about 0.0001 mg/kg to about 100 mg/kg of body weight per day. The range is more particularly from about 0.001 mg/kg to 10 mg/kg of body weight per
30 day. Even more particularly, the range varies from about 0.05 to about 1 mg/kg. Of course the dosage level will vary depending upon the potency of the particular compound. Certain

compounds will be more potent than others. In addition, the dosage level will vary depending upon the bioavailability of the compound. The more bioavailable and potent the compound, the less compound will need to be administered through any delivery route, including but not limited to oral delivery. The dosages of the human signal-transduction kinase modulators are
5 adjusted when combined to achieve desired effects. On the other hand, dosages of these various agents may be independently optimized and combined to achieve a synergistic result wherein the pathology is reduced more than it would be if either agent were used alone. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular
10 cells and conditions.

Example 1**Sequence Construction**

The novel signal transduction kinase was identified after the assembly of EST sequences from a proprietary database and the public Merck Washington University EST sequence database. The initial identification of these ESTs was performed by basic local alignment search tool (BLAST) analysis of the databases using the kinase subdomain VIB sequence HRDLKPENILLD. Sequencing of two Merck Wash. U. ESTs (zb05e11, zp83b04) provided overlapping sequencing comprised of 5'UTR, open reading frame, and 3' UTR as shown in SEQ ID NO:1. Oligonucleotide primers, sense 5'- GCCTCCATGGCCCACTCGCCGGTG-3' (SEQ ID NO:6) and antisense 5'- GGCACATGGAGCTCATGGGTTAAGC -3' (SEQ ID NO:7), were designed based on the sequence obtained from the Merck Wash U ESTs. The primers were designed to amplify a 1353bp fragment encompassing 6 bp upstream of the ATG initiation codon to 96 bp downstream of the TAA stop codon (SEQ ID NO:8). The described fragment was amplified using cDNA's derived from mRNA isolated from human lung, brain, and kidney tissue (Clontech, Palo Alto, CA), and from U937, KU812, and Jurkat cell lines using Advantage polymerase (Clontech). The PCR conditions used were: 94°C for 1 minute followed by 20 cycles of 94°C for 50 seconds, 65°C for 50 seconds, 72°C for 2 minutes. The PCR amplicons were gel purified using QiaexII agarose gel purification kit (Qiagen) and subcloned into the pCR2.1 Topo "TA" vector (Invitrogen) by the "TA" cloning approach as per the kit protocols. The topoisomerase reactions were used to transform DH5α *E.coli* competent cells (Life Technologies). Single, isolated transformed *E.coli* colonies were grown in selective media (LB broth, carbenicillin) overnight at 37°C and subsequently used to prepare plasmid DNA (Qiagen Plasmid DNA preparation kit). Clones derived from lung, brain, and kidney cDNA's were sequenced (ABI PRISM™ Dye Terminator Cycle sequencing on ABI PRISM™377 automated sequencer). This yielded single cDNA species of the novel kinase which validated the existence of this cDNA. A clone derived from lung cDNA was found to have sequence identical to that determined from the Merck Wash. U. EST clones. The 1353bp sequence (SEQ ID NO:8) was found to contain a 1248 bp (SEQ ID NO:2) open reading frame (ORF) with a Kozak consensus sequence at the initiation ATG codon.

-56-

Translation of the ORF resulted in a 416 amino acid protein sequence (SEQ ID NO:3) which contains all 12 conserved domains found in eukaryotic serine/threonine protein kinases. The protein has a predicted molecular weight of 46527.83 daltons, an isoelectric point of 5.097, and a net charge of -13 at pH 7.0.

5

Example 2**Assay For Human Kinase Activity****Generally**

Recombinant, purified GST/kinase (10 μ L) is added to 20 μ g myelin basic protein (MBP) in 10 μ L of a 3X kinase reaction buffer (KRB) containing: 60 mM HEPES (pH 7.5), 30 mM magnesium acetate, 0.15 mM ATP, 3 mM DTT, 0.03 mM sodium orthovanadate. The reaction is started by the addition of 5 μ Ci [γ -³²P] ATP (10 μ L). Samples are incubated for 5 minutes at 30°C and the reaction is stopped by addition of 4X Laemmli sample buffer. Proteins are separated on 12% Tris/glycine SDS gels, stained with Coomassie blue, dried and exposed to autoradiograph film.

15

Specific assay**3x Kinase Reaction Buffer (KRB):**

60mM HEPES, pH 7.5
30mM Magnesium Acetate
150 μ M ATP
3mM DTT
0.3mM Na₃VO₄
0.5 μ g/ml Myelin Basic Protein

20

25

ASSAY:

1. Add 10 μ L 3X KRB and 10 μ L recombinant kinase (1 μ g) to each tube.
2. Start reaction by adding 10 μ L [γ -³²P] ATP (0.5mCi/ml).
3. Incubate 30°C for 30 minutes.
- 30 4. Stop reaction with 7.5 μ L 5X Sample Buffer.
5. Boil the samples for 5 minutes then place on ice for 1 minute.

-57-

6. Load 25µL of sample (66%) on a 10-20% Tris/glycine PAGE gel and run at 35mA/gel for 47 minutes in Tris/glycine/SDS PAGE running buffer.
7. Stain the gel with Coomassie blue for 30-45 minutes then destain overnight in 10% methanol/7% acetic acid.
- 5 8. Fix the gel 1 hour in 3:1:6 MeOH/glacial acetic acid/H₂O to reduce background radiation.
9. Photograph the gel to visualize the protein bands.
10. Dry the gel and expose to film.

10 **Example 3**

Production Of Anti-kinase Polyclonal Antibodies

Antigenic peptide fragments were identified within the N-terminal, c-terminal and central regions of the novel human kinase utilizing a well established algorithm method developed by Jameson and Wolf. *The Antigenic Index: A Novel Algorithm for*

- 15 *Predicting Antigenic Determinants*, CABIOS, 4:181 (1988). The algorithm carries out six major subroutines with the following hierarchy:

- 1) determination of hydrophilicity, Hopp-Woods (1981)
- 2) calculation of surface probability, Emini (1985)
- 3) prediction of backbone or chain flexibility, Karplus-Schultz (1985)
- 20 4) prediction of secondary structure, Chou-Fasman (1978)
- 5) prediction of secondary structure, Garnier-Robson (1978)
- 6) flexibility parameters and hydropathy/solvent accessibility factors are combined to determine the antigenic index

The antigenic index was plotted for the entire molecule.

- 25 A peptide sequence, LKQQDENNASRNQA, corresponding to SEQ ID NO:3 amino acid residue positions 364-377 was selected for synthesis and antibody production based on antigenicity and uniqueness of sequence compared to other identified kinase family members. Genosys Biotechnologies, Inc. was contracted to synthesize the peptide and produce the polyclonal antisera.

- 30 Chou, P.Y. and Fasman, G.D., (1978) Prediction of the secondary structure of proteins from their amino acid sequence. *Adv. Enzymol.* 47:45-148; Emini, E. A.,

- Hughes, J., Perlow, D. and Boger, J., (1985) Induction of Hepatitis A Virus-Neutralizing Antibody by a Virus-Specific Synthetic Peptide, *J. Virology*, 55:836-839;
- Garnier, J., Osguthorpe, D.J., and Robson, B., (1978) Analysis of the accuracy and implications of simple method for predicting the secondary structure of globular
- 5 proteins, *J. Mol. Biol.*, 120:97-120; Harlow, E. and Lane, D., (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY;
- Hopp, T. P. and Woods, K. R., (1981) Prediction of Protein Antigenic Determinants from Amino Acid Sequences, *Proc. Natl. Acad. Sci.*, 78:3824-3828; Jameson, B.A., and Wolf, H., (1988) The antigenic index: a novel algorithm for predicting antigenic
- 10 determinants, *CABIOS* 4:181-186; Karplus, P.A. and Schultz, G.E., (1985) Prediction of chain flexibility in proteins, *Naturwissenschaften*, 72:212-213.

Example 4

Immunoprecipitation

- 15 Immunoprecipitation of the human kinase molecule described herein is performed substantially according to the method described by Suchard, S.J., *et al.* J. Immunol., 158:4961 (1997). Cell lysates are combined with 1 μ g of either anti-enterokinase protease cleavage site/Xpress™ antibody (Invitrogen Corp.) for the recombinant kinase described herein or peptide-specific polyclonal antibody against
- 20 the native kinase described herein. Rabbit IgG is used as a control. Samples are incubated at 4°C \geq 2 hours with rotation. Immunocomplexes are incubated with protein A Sepharose (Pharmacia) for 2 hours at 4°C with rotation. The beads are washed in buffer containing 50 mM Tris (pH 8.0), 100 mM NaCl, 1 mM Na₃VO₄, 1% Triton X-100, and Complete™ Protease Inhibitor Cocktail. Adsorbed proteins are
- 25 solubilized in sample buffer and separated on 12% SDS-PAGE minigels.

Example 5

Northern Blot Analysis

- A 289bp PCR amplicon derived from the 3' end of the open reading frame and
- 30 3'UTR region of the novel cDNA (SEQ ID NO:1 base positions 1240-1528) was obtained using the primers: sense 5' - GTCTATGATAATCACACCTGC -3' (SEQ ID

-59-

NO:9). and antisense 5'-GGCACATGGAGCTCATGGGTTAAGC-3' (SEQ ID NO:10). This DNA fragment (probe) was labeled with 50 μ Ci α -³²P-dCTP using the Ready-to-Go™ DNA Labelling Kit (Pharmacia Biotech). The labeled probe was used to hybridize against human tissue mRNA's immobilized on a solid support (Clontech Human Cancer Cell Line Multiple Tissue Northern Blot as well as a Endothelial and Epithelial Primary Cells and Cell Lines Northern Blot). Hybridization was performed essentially as described by Clontech (Protocol #PT-1200-1) using ExpressHyb™ solution. The Northern blot analysis identified a primary transcript pertaining to the novel signal transduction kinase of approximately 3.5kb in length. Prominent transcripts are apparent primarily in immune tissues (lymph node, peripheral blood leukocytes, spleen, fetal liver, bone marrow, thymus, and placenta). FIG.10.

Example 6

High Throughput Screening For Compounds Which Modulate Activity

High throughput screening for modulator compounds is performed using MBP coated 96-well FlashPlates® (NEN™ Life Science Products). Kinase reaction buffer (3X kinase reaction buffer (KRB) contains: 60 mM HEPES (pH 7.5), 30 mM magnesium acetate, 0.15 mM ATP, 3 mM DTT, 0.03 mM sodium orthovanadate) 0.25 μ Ci [γ ³³P]-ATP at a concentration no greater than 1 μ g/ml, (determined by titration of individual enzyme preparations for a concentration that allows kinetic determinations over a 1 hour time course of the human kinase) are added to each well and incubated 1 hour at 30°C in the presence or absence of 10 μ M test compound. Total reaction volume is 100 μ L. Following incubation, the reaction mixture is aspirated and the wells rinsed 2 times with 300 μ L PBS. Incorporation of radiolabeled phosphate is determined by scintillation counting, Packard Instrument Co. TopCount, 12-detector, 96-well microplate scintillation counter and luminescence counter, model B991200. Compounds which inhibit kinase activity \geq 50 percent at 10 μ M are indicated by a $>$ 50% reduction in scintillation counts. Specificity and selectivity studies is determined by titration of inhibitory compounds to determine the IC₅₀ (or other standard quantitation well known in the art for comparison) and by the substitution of other kinases in the assay. For example, determination of relative

inhibitory activity of the kinase in comparison to recombinant SOK-1 and/or mst-3, expressed and isolated in a similar manner, assayed under similar conditions, provides selectivity data.

5 **Example 7**

High Throughput Screening Protocol

Test compounds Test compounds are prepared in advance from 2.5 mg/ml stock solutions in DMSO by diluting 1:10 in distilled water and then 1:10 again. Ten (10)µl of the 1:100 dilution solutions (25 µg/ml in 1% DMSO) are prepared in 96 well
10 Microlite 1 plates (Dynatech) and plates are stored at -20°C until the evening prior to the start of the assay.

Control plates A plate containing control solutions is included in each run of the screen for QA purposes. Such plates are prepared at the beginning of the HTS campaign and stored at -20°C until required. Zero inhibition (MAX. signal) wells
15 (columns 3, 6, 8 and 10) contain 10 µl of 1% (v/v) DMSO solution in MilliQ water. 100% inhibition (MIN signal) wells (columns 1, 4, 9 and 11) contain 10 µl of 220 nM ZM333141/1 in 1% DMSO solution in MilliQ water. 50% inhibition (REF. signal) wells (columns 2, 5, 7 and 12) contain a reference compound at a concentration known to provide approximately 50% inhibition in 1% (v/v) DMSO solution in MilliQ water.

20 **Assay components**

(1) recombinant kinase (expressed in *E.coli* or eukaryotic cells as described herein) or a lysate of a prokaryotic or eukaryotic cell expressing recombinant enzyme, or the natural enzyme partially purified from a human cell line.

(2) [γ -³³P]-adenosine triphosphate

25 (3) myelin basic protein linked to the surface of PVT SPA beads (purchased from Amersham International) by an antibody-protein A or other appropriate method.

To Microlite 1 plates containing 10µl of test compound, which have been left on the bench overnight to reach room temperature, 25µl of GST-Rb/ATP/ATP³³ is added, immediately followed by 20µl of Enzyme, using two Multidrops. The plates are
30 stacked in 13 plate stacks (with an empty plate on top of each stack to minimise

-61-

evaporation from the top plate) and left at room temperature for 105 minutes. 150µl of "Stop Solution" containing beads antibody and EDTA is added using a Multidrop. The plates are sealed with Topseal-S plate sealers and left on the bench overnight, surrounded by Scotlab perspex screens. The plates are then centrifuged (Heraeus
5 Megafuge 3.0R) at 2500rpm, 1124xg., for 5 minutes (2 plates per trunnion) and counted on a Topcount (I4.34); (isotope:P³³; counting time: 20 seconds/well).

The data may be analysed using well-known software systems. A threshold for inhibition is set. e.g., 60% inhibition of scintillation signal. Compounds reaching the inhibition threshold are scored as active.

10

Example 8

Pcr Of Seq Id No:1 From Various Human Cdnas Of Hematopoietic Origin

SEQ ID NO:1 message is determined to be present in other cells of hematopoietic origin by PCR with cDNA's isolated from different cell-lines of
15 hematopoietic origin.

Target product was amplified from 2-2.5 ng of reverse transcribed mRNAs in a 20 µL reaction using Advantage™ KlenTaq polymerase (Clontech # 8417-1, lot 7020348) according to the manufacturer's recommendations. Primer set sequences are sense 5'- GTCTATGATAATCACACCTGC -3' (SEQ ID NO:9), and antisense 5'-
20 GGCACATGGAGCTCATGGGTAAAGC -3' (SEQ ID NO:10). A 289bp PCR amplicon indicative of expression of the novel kinase is generated derived from the 3' end of the open reading frame and 3'UTR region of the novel cDNA (SEQ ID NO:1 base positions 1240-1528). The PCR conditions used are: 94°C for 1 minute followed by 20 cycles of 94°C for 50 seconds, 65°C for 50 seconds, 72°C for 2 minutes. 10 µL
25 of each reaction is analyzed on a 1% agarose gel containing 0.5 µg/mL final concentration ethidium bromide in TAE buffer as in Sambrook *et al.*, Molecular Cloning Lab Manual. Second Edition, Cold Spring Harbor Press (1989), using 600 ng of 1kb DNA ladder as markers (Life Technologies, cat # 15615-016).

30

Example 9**Expression And Purification Of Gst/Human Signal Transduction Kinase Fusion Protein**

A single, isolated, BL21 transformed clone was grown overnight in 10mL Lennox L broth (LB broth containing 50mg/ml carbenicillin) and then seeded into 1 liter LB broth/carbenicillin and grown at 37°C with shaking (225rpm) to an A_{600} of 0.5-0.8. Expression of GST/kinase fusion protein was induced by adding isopropylthio- β -galactoside to 100mM and continuing the incubation for 2 additional hours. Following incubation, the cells were centrifuged 1500 x g for 10 min at 4°C, resuspended in 50mL phosphate buffered saline (PBS) containing Complete™ Protease Inhibitor Cocktail (Boehringer Mannheim GmbH), then lysed by sonication on ice. Triton X-100 was added to the sonicate to a final concentration of 1% to aid in the solubilization of the fusion protein. Cellular debris was removed by centrifugation (12,000 x g, 4°C) and the supernatant was used as the source for obtaining purified GST/kinase.

Purification of gst/kinase fusion protein

GST/kinase was purified by Glutathione Sepharose 4B beads (Pharmacia) affinity column chromatography using a 1ml gravity fed open column. The suspension was allowed to pass through the column then the column was washed three times with PBS containing protease inhibitors. Finally, the GST-kinase fusion protein was eluted from the column by the addition of 1mL elution buffer (10mM reduced glutathione in 50mM Tris-HCl, pH 8.0 with protease inhibitors). The GST/kinase was stored in aliquots at -20°C until needed.

Example 10**Electroporation**

Note: All of the steps in this procedure, with the exception of the application of the pulse, are carried out under sterile tissue culture conditions.

1. Grow cells to a density of 80% confluency. Harvest by immersing cells in phosphate buffered saline (PBS) for 5 minutes. Detach from flask by pipetting PBS over the cells.

-63-

Determine the cell number using a hemocytometer. Cells are pelleted by centrifugation at 1500 rpm in a benchtop centrifuge. Wash cells once with PBS and pellet by centrifugation.

2. Resuspend cells at a density of 4×10^6 cells/500 μ l media without fetal bovine serum (FBS).
- 5 3. Under a tissue culture laminar flow hood remove the required number of 0.4 cm cuvette from the individual sealed packages. Remove the caps and add 500 μ l of resuspended cells to the cuvette.
4. Resuspend 10-20 μ g of DNA at a concentration of 1 to 2 μ g/ μ l in sterile water or TE (10 mM Tris pH 7.5, 1 mM EDTA) for each electroporation.
- 10 5. Add DNA to cuvette and gently tap several times to mix. Replace the caps on the cuvettes and incubate on ice for 10 minutes.
6. Following the 10 minute incubation, begin electroporation. Gently flick the cuvette a few times to resuspend any cells that may have settled, then apply the pulse. Following pulse, incubate cuvette at room temperature for an additional 10 minutes.
- 15 7. Cells are plated following the second 10 minute incubation period. Remove the caps from the cuvette, under a tissue culture laminar flow hood, and add 1 ml of complete media. Gently aspirate the cells from the cuvette using a sterile Pasteur pipette and add to a 100 mm dish containing 9 ml of complete media. Incubate overnight at 37°C with 5% CO₂.
- 20 8. Change media after 16–24 hours.
9. Harvest cells 48-72 hours after electroporation and/or begin selection for stable transformants by the addition of the appropriate antibiotic whose resistance is conferred by the expression vector transfected into the cells (e.g. neomycin (G418), hygromycin, Zeocin™ (Invitrogen)).
- 25 10. Stable cell lines are selected over a period of 14-21 days in the presence of the antibiotic at a concentration determined to result in cell death of untransfected cells within 3-5 days. During the selection process, the media is changed every 3-4 days to maintain the concentration of antibiotic in the media. After selection, clonal stable cell lines are isolated and expanded by placing into separate tissue culture vessels.

Example 11**Lipid Mediated Transfection (Life Technologies, Gaithersburg, Md, Lipofectin Kit)**

1. Seed $1-2 \times 10^5$ cells/60-mm tissue culture plate in 4 ml of the appropriate growth medium supplemented with serum.
- 5 2. Incubate the cells at 37°C in a CO₂ incubator for 18-24 h. Cells should be 30-50% confluent.
3. Prepare the following solutions in 12 x 75 mm sterile tubes:
Solution A: For each transfection dilute 2 mg DNA into 100ml OPTI-MEM I Medium.
Solution B: For each transfection dilute 5-20 µl of LIPOFECTIN Reagent into 100 ml
10 OPTI-MEM I Medium, and allow to stand at room temperature for 30-45 min.
4. Combine the two solutions, mix gently, and incubate at room temperature for 10-15 min.
5. Wash cells once with 2 ml of serum-free growth medium without antibacterial agents.
- 15 6. Add 1.8 ml serum-free growth medium to each tube containing the LIPOFECTIN Reagent-DNA complexes. Mix gently and overlay onto cells. (Note: Cells may be transfected in the presence of serum, see "Transfection in the presence of serum" section below.)
7. Incubate the cells for 5-24 h at 37°C in a CO₂ incubator.
- 20 8. Replace the DNA containing medium with 4 ml of growth medium supplemented with the normal percentage of serum and incubate the cells at 37°C in a CO₂ incubator for another 48 h.
9. Subculture cells at desired ratios, at least 1:5, into selection medium.

25 Transfection in the presence of serum

Successful transfection with LIPOFECTIN Reagent may be achieved in the presence of serum providing that lipid-DNA complexes are formed in serum free medium (Note: steps 1-4 in procedures above). Antibacterial agents should not be added to cells in the medium together with the DNA-lipid complexes. Dulbecco's
30 Modified Eagle Medium (Life Technologies, Cat. No. 11960), OPTI-MEM I Reduced

Serum Medium, is recommended, or other serum-free growth medium supplemented with 5-10% fetal bovine serum.

Example 12

5 Dominant Negative/Inactive Kinase Mutant

A dominant negative/inactive kinase mutant, for example, can readily be produced with the tools provided herein to further produce one embodiment exemplified herein by changing the lysine at SEQ ID NO:3 position 53 to an arginine. This mutation has been shown to produce inactive and/or dominant negative kinases
10 for numerous other serine/threonine kinases. *See, e.g., Hanks, S.K., et al. Science, 241:42 (1988).* A dominant negative or inactive kinase mutant of the novel kinase was produced by changing the lysine at position 53 to an arginine. The mutation was introduced into the novel kinase/pCR2.1 construct using the Quick Change Site Directed Mutagenesis Kit (Stratagene) and the oligonucleotides 5'-
15 CAGCAAGTCGTTGCTATCCGGATCATAGACCTTGAG -3' (sense) (SEQ ID NO:13) and 5'-CTCAAGGTCTATGATCCGGATAGCAACGACTTGCTG -3' (antisense) (SEQ ID NO:14). This produced the construct K53R/pCR2.1. Epitope tagged dominant negative vector construction: A constitutive expression construct containing the novel kinase K53R dominant negative kinase CDS was produced using
20 pcDNA3.1His (Invitrogen Corporation). This expression construct is designed to express an N-terminal epitope tagged fusion protein containing the following protein sequence: Methionine - (Histidine)₆ - Enterokinase protease cleavage site/XpressTM antibody epitope - novel kinase K53R dominant negative kinase protein. The novel kinase K53R cDNA was excised from the K53R/pCR2.1 construct using EcoRI (5')
25 and EcoICRI (3') and inserted into the pcDNA3.1His vector using EocRI (5') and EcoRV (3') restriction endonuclease sites at the 5' end and 3' end, respectively.

Example 13**Identification Of The Mouse Homolog Of The Human Ser/Thr Kinase (SEQ ID NO:3)**

RT-PCR analysis of MEL (mouse erythroleukemia cell line) was performed using primers specific to the 5' and 3' end of the human SEQ ID NO:3 coding region (FIG.7: SEQ ID NO:6 and SEQ ID NO:7, respectively). The conditions are outlined in Example I. The resulting 1350bp PCR amplicon was gel purified, ligated into pCR2.1 TOPO, and sequenced as outlined in Example I. This sequence was used to identify mouse EST clones in the Merck Wash U database by BLAST analysis. The mouse EST clone, vx21d04 (accession # AA881667), was identified as the mouse ortholog of the human TEN-1 kinase based on the sequence similarity to SEQ ID NO:3 versus SOK1 and MST3. The clone was obtained and sequenced in its entirety:

Murine signal transduction kinase (SEQ ID NO:3 variant):

Molecular Weight 46612.94 Daltons
 416 Amino Acids; 51 Strongly Basic(+) Amino Acids (K,R); 66 Strongly Acidic(-) Amino Acids (D,E); 136 Hydrophobic Amino Acids (A,I,L,F,W,V); 103 Polar Amino Acids (N,C,Q,S,T,Y); 5.054 Isoelectric Point ; -14.012 Charge at PH 7.0

Example 14**Activation Of Transcription Factors By TEN-1 Kinase**

Shown below is the general format of the transcription activation reporter assays used to determine if the signal transduction kinase (SEQ ID NO:3) activates specific transactors. The PathDetect™ System (including reporter constructs and positive control constructs) was used to perform these assays. Stratagene, La Jolla, CA. All assays were performed in triplicate and repeated several separate times.

1. The cells were diluted to 1.5×10^5 /ml and 2ml (3×10^5 cells) were plated into each well of 6-well dishes
2. The cells were cultured overnight at 37°C, 5% CO₂, 90% relative humidity (these conditions were used throughout the assay)

-67-

3. Cells were transfected using Stratagene's Lipotaxi reagent according to the protocol supplied with the reagent. The protocol is as follows (described for 3 transfections)
4. Combine 840µl DMEM + 60µl Lipotaxi reagent in a polystyrene tube
5. Add DNA's (see table) to the DMEM/Lipotaxi solution
6. Incubate for 15-30 minutes at room temperature
7. Add 2.1ml DMEM to the DMEM, Lipotaxi, DNA solution
8. Remove the culture media from the cells
9. Add 1ml of the Lipid/DNA solution to each well of cells in a drop wise fashion
- 10 while swirling the dish
10. Incubate the Lipid/DNA:cell culture for 5-7 hours at 37°C in 5% CO₂
11. Add 1ml DMEM/ 1% FBS and culture overnight
12. Replace the media with fresh DMEM/0.5% FBS and culture overnight
13. Test the cells for luciferase activity using Packard's Luc-Lite kit reagents and
- 15 protocol as follows
14. Remove the media from the cells and add 300µl of Luc-Lite reagent that was previously diluted 1:1 with PBS containing 1mM CaCl₂ and MgCl₂
15. Incubate at room temperature for 10 minutes
16. Dispense 200µl of the cell lysate into white plastic 96-well plates and read the
- 20 light emission on a Perkin-Elmer/ Tropix 96-well Fluorimeter

Table of DNA's used in a PathDetect™ assay (TEN1 = SEQ ID NO:3)

Reporter Vector @ 1µg/well	Positive Control Vector, @ 50ng/well	Kinase Expression Vector @ 50ng, 250ng, 500ng, 750ng/well	Expression Vector w/o Insert @ 50ng, 250ng, 500ng, 750ng/well	Carrier Vector DNA @1µg/well
pX-Luc, 3µg, 3µl	---	---	---	3µg, 3µl
pX-Luc, 3µg, 3µl	---	---	pIRES-EGFP, 150ng, 3µl	2.85µg, 2.85µl
pX-Luc, 3µg, 3µl	---	---	pIRES-EGFP, 750ng, 3µl	2.25µg, 2.25µl
pX-Luc, 3µg, 3µl	---	---	pIRES-EGFP, 1500ng, 3µl	1.5µg, 1.5µl
pX-Luc, 3µg, 3µl	---	---	pIRES-EGFP, 2250ng, 3µl	0.75µg, 0.75µl
pX-Luc, 3µg, 3µl	---	TEN1#1/pIRES-EGFP, 150ng, 3µl	---	2.85µg, 2.85µl

Reporter Vector @ 1µg/well	Positive Control Vector, @ 50ng/well	Kinase Expression Vector @ 50ng, 250ng, 500ng, 750ng/well	Expression Vector w/o Insert @ 50ng, 250ng, 500ng, 750ng/well	Carrier Vector DNA @ 1µg/well
2.25µg, 2.25µl	---	TEN1#1/pIRES-EGFP, 750ng, 3µl	---	
pX-Luc, 3µg, 3µl	---	TEN1#1/pIRES-EGFP, 1500ng, 3µl	---	1.5µg, 1.5µl
pX-Luc, 3µg, 3µl	---	TEN1#1/pIRES-EGFP, 2250ng, 3µl	---	0.75µg, 0.75µl
pX-Luc, 3µg, 3µl	pFC-X, 150ng, 6µl		---	2.85µg, 2.85µl

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

CLAIMS:

What is claimed is:

1. A purified polynucleotide comprising a nucleic acid sequence encoding the polypeptide having the sequence substantially as depicted in SEQ ID NO:3 or a
5 pharmacologically active and/or biologically active functional derivative thereof.
2. The polynucleotide of Claim 1 wherein the polynucleotide sequence comprises the sequence substantially as depicted in SEQ ID NO:2.
- 10 3. An expression vector comprising the polynucleotide of Claim 1.
4. An antisense molecule comprising the complement of the polynucleotide of Claim 2 or a biologically-effective portion thereof.
- 15 5. A host cell transformed with the expression vector of Claim 3.
6. A purified polypeptide comprising the amino acid sequence substantially as depicted in SEQ ID NO:3.
- 20 7. An antibody specific for the polypeptide of Claim 6.
8. A method for producing cells which express a biologically polypeptide substantially as depicted in SEQ ID NO:3 or a pharmacologically active and/or biologically active functional derivative thereof., said method comprising
25 a) culturing a host cell according to Claim 5 under conditions suitable for the expression of said polypeptide.
9. A method for producing a polypeptide having the amino acid sequence substantially as
30 depicted in SEQ ID NO:3, said method comprising the steps of:

-70-

- a) culturing a host cell according to Claim 5 under conditions suitable for the expression of said polypeptide, and;
- b) recovering said polypeptide from the host cell culture.

5 10. A method of identifying compounds that modulate the activity of a signal-transduction kinase, comprising:

- (a) combining a candidate compound modulator of a signal-transduction kinase activity with a polypeptide of a signal-transduction kinase molecule having a sequence substantially as depicted in SEQ ID NO:3, and;
- 10 (b) measuring an effect of the candidate compound modulator on the activity.

11. A method of identifying compounds that modulate the activity of a signal-transduction kinase, comprising:

15

- (a) combining a candidate compound modulator of a signal-transduction kinase activity with a host-cell expressing the polypeptide of a signal-transduction kinase molecule having a sequence substantially as depicted in SEQ ID NO:3, and
- (b) measuring an effect of the candidate compound modulator on the activity.

20

12. A compound that modulates the activity of a signal-transduction kinase identified by the method of Claim 10.

13. A compound that modulates the activity of a signal-transduction kinase identified by
25 the method of Claim 11.

14. A pharmaceutical composition comprising a compound that modulates the activity of signal-transduction kinase identified by the method of of Claim 10.

30 15. A method of treatment of a patient in need of such treatment for a condition which is

mediated by a signal-transduction kinase, comprising administration of a signal-transduction kinase modulating compound according to Claim 12.

16. A method of treatment of a patient in need of such treatment for a condition which is mediated by a signal-transduction kinase, comprising administration of a signal-transduction
5 kinase modulating compound according to Claim 13.

17. A method for inhibiting the expression of a signal-transduction kinase in a cell comprising administering an effective amount of the antisense molecule of Claim 4 to said cell.

10

18. A diagnostic composition for the identification of a polypeptide sequence comprising the amino acid sequence substantially as depicted in SEQ ID NO:3 comprising the antibody of Claim 7.

- 15 19. A diagnostic composition for the identification of a polynucleotide sequence comprising the sequence substantially as depicted in SEQ ID NO:2 comprising the PCR primers derived from SEQ ID NO:2.

1/18

FIG.1

5'
TAACAGCCCACCTCCTAGCCCCGGGCTACGCGCCGCCAGCCCAGTAACCCCACTTTTGTGTGTCTCCAGGCCC
CGATCGAAAAGCCTGGGAGGGCCCGGAACTACCCCGGAGGGAGGAGCCAGTCCGAACCCAAGGCGCCACCGCC
5 GCAGAAGCGGAGCGAGGCAGCATTGCGCTCCATGGCCCACTCGCCCGTGGCTGTCCAAGTGCCTGGGATGCAGAA
TAACATAGCTGATCCAGAAGAACTGTTCAAAAATTAGAGCGCATTGGGAAAGGCTCATTTGGGGAAGTTTCAA
AGGAATTGATAACCGTACCCAGCAAGTCGTTGCTATTAAATCATAGACCTTGAGGAAGCCGAAGATGAAATAGA
AGACATTCCAGCAAGAAATAACTGTCTTGAGTCAATGTGACAGCTCATATGTAACAAAATACTATGGGTCATATTT
AAAGGGGTCTAAATTATGGATAATAATGGAATACCTGGGCGGTGGTTCAGCACTGGATCTTCTTCGAGCTGGTCC
10 ATTTGATGAGTTCCAGATTGCTACCATGCTAAAGGAAATTTTAAAGGTCTGGACTATCTGCATTTCAGAAAAGAA
AATTCACCSAGACATAAAAGCTGCCAATGTCTTGCTCTCAGAACAAGGAGATGTTAACTTGCTGATTTTGGAGT
TGCTGGTCAGCTGACAGATACACAGATTAAAAGAAATACCTTTGTGGGAAGTCCATTTTGGATGGCTCCTGAAGT
TATTCACAGTCAGCTTATGACTCAAAGCTGACATTTGGTCATTGGGAATTACTGCTATTGAACTAGCCAAGGG
AGAGCCACCTAACTCCGATATGCATCCAATGAGAGTTCTGTTTCTTATCCCAAAAACAATCCTCCAACCTCTTGT
15 TGGAGACTTTACTAAGTCTTTTAAAGGAGTTTATTGATGCTTGCTGAACAAAGATCCATCATTTCTGCTCTACAGC
AAAAGAATCTCTGAAACACAAATTCATTGTAAAAAATTCAAAGAAGACTTCTTAICTGACTGAAGTATAGATCG
TTTTAAGAGATGGAAGGCAGAAGGACACAGTGATGATGAATCTGATTCCGAGGGCTCTGATTCCGAATCTACCAG
CAGGGAAAACAATACTCATCCTGAATGGAGCTTTACCACCGTACGAAAGAAGCCTGATCCAAAGAAAGTACAGAA
TGGGGCAGAGCAAGATCTTGTGCAAACCYTGAGTTGTTTGTCTATGATAATCACACCTGCATTTGCTGAAGTTAA
20 ACAGCAGGACGAGAATAACGCTAGCAGGAATCAGGCGATTGAAGAACTCGAGAAAAGTATTGCTGTGGCTGAAGC
CGCCTGTCCCGGCATCACAGATAAAATGGTGAAGAACTAATTGAAAAATTTCAAAGTGTTCAGCAGACGAATC
CCCCTAAGAACTTATTATTGGCTTCTGTTTCATATGGACCCAGAGAGCCCCACCAAACCTACGTCAAGATTAAC
AATGCTTAACCCATGAGCTCCATGTGCCTTTTGGATCTTTGCAACACTGAAGATTTGGAAGAAGCTATTAAACTA
TTTGTGATGGCSTTTATCATTTTATATTTTGAAAGGATTATTTTGTAAGGAATAACTTTTAATACTATAGTTTC
25 ACCTGTATTCTAGTAAATGTTGAGACACCGTTTIGCTTTTAAAGTATCCCTATTTCTTAAGTTACGAGGATGAATA
CCTTTTCAATTTTGATCTTTAGTTGACTCTACAGTCATGAAACATACAGGTCTTTCAAAGTCATTCTCAATATTC
AGCTTTTGTAAATTATCAAGCTTCAAAGGCTTTTTTTTTAAAAAACAATGCATATTCTAAAAATGACTATT
GGGTGGGAGGTGTAAATAAGTCATACCTTCTTAAACAGAAAATTTAAGTAAAGTCTTTTAAATGAAACCTGTA
AAAGTATTGACTCTTCTACCAAGTTGGTATGATATTCCAGGCAGCTCAATGATTATCACATTTGAGACCTGTGT
30 TTGAAGCATTTACAGGCAATGTACAGCAACAGAGGTACCTCTTGGTGTATAGTATTTACATTCCTTTTAGGTAG
AAGAGGCAATTTTACCTTATTTTACATGGTTAGAAATTTAAAGCAAGATCATTTACCCAAGGATAGGTGTTTGG
TAATGTTGAAGGAGTTAGTCTGGCTTCATGTTTTACATCTTCAACTAAAATCCCACTATCTGCTTGGATTTGG
AGAGCCAAAAATAAAGCTGATTGTCATGTGATTAAATATCTGATCAACAGGTATGAATATAACTTAAATCAGCA
TATTTTGGCATGGAATAAATTGCTCTATAAACTATTTATATATTTTGTCTTCTATAATTATCACTAATAAGC
35 ATCAGTTTGTGTTTTTAAAGGATATTTAAGTGAGCATTTTCTAGTTCATATGAAAAATAACCATAGTACAGGAT
GATTTCTGTCCACACAAAGGTTAAATTAGATTGCACAGTTAATTTTCACTTATATTTATGGTACTATTATGTGGG
TGATGCCTTTTCTTTTAAAGCCAGTACATATATTATGCCTGCCTAAGTTCTGAACTGGGGCTGTATTTTCAGTAG
TTGTAGAAATTATTGATATTTAGTTTTGATAGCTAATGTTTAAATTGTTTGGATCTGCACAGTTTGGTTTTTGCACA
AAAGTCATTTAAAAAATCTGAGTAATTGTCAAATATTAAAGAAAGATATTCTTCTGTAAAGGAATACAGTTTTT
40 TAGTCAAAGTGGCCATTACATCCTCTTTTTAATTTACATAATACAGATACTTGAGAAAGTTGTTGTGGTGTGTA
TGCCAAAGAAATCTTTTTTATTGGTGCCTATATTGTAACAATTAATTTTAAATGCATTGTATTTGAAGTAACGGT
TCAGTTAAATTTTCACTGCTGTGTAACTGAAACACAATTACAGTTTATAATCATCTGTAGAAGTCTGGAGATA

2/18

FIG 1 (continued)

ATTTTGCAACTCATGTTATGGGTTAAATGAATATTTTGTAAAAGTAAAAGCAACAAATTTATAAATTGATTATT
5 TGAAACTTTACAACACAATTGCATCCCAAATACAAATTGTATTGCTTATTCATTATAGCTATTCGTCCTGTAATC
TGITTTCTAGGTGAAGCATACTCCAGTGTTTTAGGGGTTTTGAAAATAAATA 3'

SEQ ID NO:1

10

3/18

FIG.2

5'

ATGGCCCACTCGCCGGTGGCTGTCCAAGTGCCTGGGATGCAGAATAACATAGCTGATCCAGAAGAACTGTTTACA
AAATTAGAGCGCATTGGGAAAGGCTCATTGGGGAAGTTTTCAAAGGAATTGATAACCGTACCCAGCAAGTCGTT
5 GCTATTAAAAATCATAGACCTTGAGGAAGCCGAAGATGAAATAGAAGACATTCAGCAAGAAATAACTGTCTTGAGT
CAATGTGACAGCTCATATGTAAACAAAATACTATGGGTTCATATTTAAAGGGGTCTAAATTATGGATAATAATGGAA
TACCTGGGCGGTGGTTTCAGCACTGGATCTTCTTCGAGCTGGTCCATTTGATGAGTTCCAGATTGCTACCATGCTA
AAGGAAATTTTAAAAGGTCTGGACTATCTGCATTCAGAAAAGAAAATTCACCGAGACATAAAAAGCTGCCAATGTC
TTGCTCTCAGAACAAGGAGATGTTAAACTTGCTGATTTTGGAGTTGCTGGTCAGCTGACAGATACACAGATTAAA
10 AGAAATACCTTTGTGGGAACTCCATTTTGGATGGCTCCTGAAGTTATTCAACAGTCAGCTTATGACTCAAAGCT
GACATTTGGTTCATTGGGAATTACTGCTATTGAACTAGCCAAGGGAGAGCCACCTAACTCCGATATGCATCCAATG
AGAGTTCTGTTTCTTATTTCCAAAAACAATCCTCCAACCTCTTGTGGAGACTTTACTAAGTCTTTTAAGGAGTTT
ATTGATGCTTGCTGAACAAAGATCCATCATTTCTGCTACAGCAAAAGAACTTCTGAAACACAAATTCATTGTA
AAAAATTCAAAGAAGACTTCTTATCTGACTGAACTGATAGATCGTTTTAAGAGATGGAAGGCAGAAGGACACAGT
15 GATGATGAATCTGATTCCGAGGGCTCTGATTCCGAATCTACCAGCAGGGAAAACAATACTCATCCTGAATGGAGC
TTTACCACCGTACGAAAGAAGCCTGATCCAAAGAAAGTACAGAATGGGGCAGAGCAAGATCTTGTGCAAACCCCTG
AGTTGTTTGTCTATGATAATCACACCTGCATTTGCTGAACTTAAACAGCAGGACGAGAATAACGCTAGCAGGAAT
CAGGCGATTGAAGAACTCGAGAAAAGTATTGCTGTGGCTGAAGCCGCCTGTCCCGGCATCACAGATAAAATGGTG
AAGAAACTAATTGAAAAATTTCAAAGTGTTCAGCAGACGAATCCCCCTAA 3'

20

SEQ ID NO:2

4/18

FIG.3

MAHSPVAVQVPGMQNNIADPEELFTKLERIGKGSFGEVFKGIDNRTQQVVAIKIIDLEEAEDEIEDIQ
QEITVLSQCDSSYVTKYYGSYLKGSKLWIIMEYLGGGSSALDLLRAGPFDEFQIATMLKEILKGLDYLH
5 SEKKIHRDIKAANVLLSEQGDVKLADFGVAGQLTDTQIKRNTFVGTPFWMAPEVIQQSAYDSKADIWS
LGITAIELAKGEPPNSDMHPMRVLFLLPKNNPPTLVGDFTKSFKEFIDACLNKDPSFRPTAKELLKHK
FIVKNSKKTSYLTELIDRFKRWKAEGHSDDSDSEGSSESTSRENNTHPEWSFTTVRKKPDPKKVQN
GAEQDLVQTLSCLSMIITPAFAELKQQDENNASRNAIEELEKSIAVAEEAACPGITDKMVKKLIEKFQ
KCSADESP

10

SEQ ID NO:3

5/18

FIG.4

MAHLRGFANQHSRVDPEELFTKLDRIGKGSFGEVYKGIDNHTKEVVAIKIIDLEEADEIEDIQQEITVLSQCDS
PYITRYFGSYLKSTKLWIIIMEYLGGGSSALDLLKPGPLEETYIATILREILKGLDYLHSEKIHARDIKAANVLLSE
5 QGDVKLADFGVAGQLTDTQIKRNTFVGTPFWMAPEVIKQSAYDFKADIWSLGITAIELAKGEPPNSDLHPMRVLF
LIPKNSPPTLEGQHSKPFKEFVEACLNKDPRFRPTAKELLKHKFITRYTKKTSFTELIDRYKRWKSEGHGEES
SEDSIDIDGEAEDGEQGPIWTFPPTIRPSPHSKLHKGTALHSSQKPADAVKRQPRSQCCLSTLVRPVEGELKEKHKQ
SGGSVGALEELENAFSLAEESCPGISDKLMVHLVERVQRFSHNRNHLTSTR

10

SEQ ID NO:4

6/18

FIG.5

MAHSPVQSGLPGMQNLKADPEELFTKLEKIGKGSFGEVFKGIDNRTQKVVAIKIIDLEEADEIEDIQQEITVLS
QCDSPYVTKYYSYLKDTKLWIIMEYLGSGSALDLLEPGPLDETQIATILREILKGLDYLHSEKKIHRDIKAANV
5 LLSEHGEVKLADFGVAGQLTDTQIKRNTFVGTPFWMAPEVIKQSAYDSKADIWSLGITAIELARGEPPHSELHPM
KVLFLIPKNNPPTLEGNYSKPLKEFVEACLNKEPSFRPTAKELLKHKFILRNAKTSYLTTELIDRYKRWKAEQSH
DDSSSESDAETDGQASGGSDSGDWIFTIREKDPKNLENGALQPSDLDRNKMKDIPKRPFSQLSTIISPLFAEL
KEKSQACGGNLGSIEELRGAIYLAEEVCPGISDTMVAQLVQRLQRYSLSGGGTSSH

10

SEQ ID NO:5

7/18

FIG. 6

```

vx21d04 MAHSPPVAVQVP G M Q N N I A D P E E L F T K L E R I G K G S F G E V F K 40
hten1 MAHSPPVAVQVP G M Q N N I A D P E E L F T K L E R I G K G S F G E V F K 40
hmst3 MAHSPPVAVQVP G M Q N N I A D P E E L F T K L E R I G K G S F G E V F K 40
hsok1 MAHSLRSGFAN - - - QHSRVDPPEELFTKLDIRIGKGSFGGEVYK 36

vx21d04 GIDNRTQQVVVAIKIIDLLEAEDEIEDIQQEITVLSQCCDS 80
hten1 GIDNRTQQVVVAIKIIDLLEAEDEIEDIQQEITVLSQCCDS 80
hmst3 GIDNRTQQVVVAIKIIDLLEAEDEIEDIQQEITVLSQCCDS 80
hsok1 GIDNRTQQVVVAIKIIDLLEAEDEIEDIQQEITVLSQCCDS 76

vx21d04 YVTKYYGSSYLKSSKLWIMEYLGSSGSSALDLDLRAGPFDSEFQ 12
hten1 YVTKYYGSSYLKSSKLWIMEYLGSSGSSALDLDLRAGPFDSEFQ 12
hmst3 YVTKYYGSSYLKSSKLWIMEYLGSSGSSALDLDLRAGPFDSEFQ 12
hsok1 YVTKYYGSSYLKSSKLWIMEYLGSSGSSALDLDLRAGPFDSEFQ 11

vx21d04 IATMLKEILKGLDYLSSEKKIHRDIAKANVLLSEQGGDVKL 16
hten1 IATMLKEILKGLDYLSSEKKIHRDIAKANVLLSEQGGDVKL 16
hmst3 IATMLKEILKGLDYLSSEKKIHRDIAKANVLLSEQGGDVKL 16
hsok1 IATMLKEILKGLDYLSSEKKIHRDIAKANVLLSEQGGDVKL 15

vx21d04 ADFGVAGQLTDTOIKRNTFVGTFFWMAPEVIOQSSAYDSKA 20
hten1 ADFGVAGQLTDTOIKRNTFVGTFFWMAPEVIOQSSAYDSKA 20
hmst3 ADFGVAGQLTDTOIKRNTFVGTFFWMAPEVIOQSSAYDSKA 20
hsok1 ADFGVAGQLTDTOIKRNTFVGTFFWMAPEVIOQSSAYDSKA 19

vx21d04 DIWSLGGITAEELAKGEPNNSDMHMPMRVLELIPKNNPPTLE 24
hten1 DIWSLGGITAEELAKGEPNNSDMHMPMRVLELIPKNNPPTLE 24
hmst3 DIWSLGGITAEELAKGEPNNSDMHMPMRVLELIPKNNPPTLE 24
hsok1 DIWSLGGITAEELAKGEPNNSDMHMPMRVLELIPKNNPPTLE 23

vx21d04 GDFTKSFKKEFIDACLNKDPSPFRPTAKELLKHKFFIVKNSKK 28
hten1 GDFTKSFKKEFIDACLNKDPSPFRPTAKELLKHKFFIVKNSKK 28
hmst3 GDFTKSFKKEFIDACLNKDPSPFRPTAKELLKHKFFIVKNSKK 28
hsok1 GDFTKSFKKEFIDACLNKDPSPFRPTAKELLKHKFFIVKNSKK 27

vx21d04 TSYLETELDIDREFKRWKAECHSDDESSEGSSESSSRES 31
hten1 TSYLETELDIDREFKRWKAECHSDDESSEGSSESSSRES 31
hmst3 TSYLETELDIDREFKRWKAECHSDDESSEGSSESSSRES 31
hsok1 TSYLETELDIDREFKRWKAECHSDDESSEGSSESSSRES 31

vx21d04 NPHPEWSFTTVRKKEPDPKKLQNGE - - - - - EQDLVQTL 35
hten1 NPHPEWSFTTVRKKEPDPKKLQNGE - - - - - EQDLVQTL 35
hmst3 NPHPEWSFTTVRKKEPDPKKLQNGE - - - - - EQDLVQTL 35
hsok1 NPHPEWSFTTVRKKEPDPKKLQNGE - - - - - EQDLVQTL 35

vx21d04 - - - - - CLSMIITPAFAELKQODENNASRNQAIEELEKS 38
hten1 - - - - - CLSMIITPAFAELKQODENNASRNQAIEELEKS 38
hmst3 - - - - - CLSMIITPAFAELKQODENNASRNQAIEELEKS 38
hsok1 - - - - - CLSMIITPAFAELKQODENNASRNQAIEELEKS 39

vx21d04 AVAEACPGITDKMVKKLIERFQKCS - - - - - ADESP 41
hten1 AVAEACPGITDKMVKKLIERFQKCS - - - - - ADESP 41
hmst3 AVAEACPGITDKMVKKLIERFQKCS - - - - - ADESP 41
hsok1 AVAEACPGITDKMVKKLIERFQKCS - - - - - ADESP 42

```

Decoration 'Decoration #1': Box residues that match vx21d04 exactly.

8/18

FIG.7

5' GCCTCCATGGCCCACTCGCCGGTG 3'

5

SEQ ID NO:6

5' GGCACATGGAGCTCATGGGTAAAGC 3'

10

SEQ ID NO:7

9/18

FIG.8

5'

GCCTCCATGGCCCACTCGCCGGTGGCTGTCCAAGTGCCTGGGATGCAGAATAACATAGCTGATCCAGAAGAACTG
TTCACAAAATTAGAGCGCATTGGGAAAGGCTCATTGGGGGAAGTTTTCAAAGGAATTGATAACCGTACCCAGCAA
5 GTCGTTGCTATTAAAATCATAGACCTTGAGGAAGCCGAAGATGAAATAGAAGACATTGAGCAAGAAATAACTGTC
TTGAGTCAATGTGACAGCTCATATGTAACAAAATACTATGGGTTCATATTTAAAGGGGTCTAAATTAIGGATAATA
ATGGAATACCTGGGCGGTGGTTTCAGCACTGGATCTTCTTCGAGCTGGTCCATTTGATGAGTTCCAGATTGCTACC
ATGCTAAAGGAAATTTTAAAGGTCTGGACTATCTGCATTGAGAAAAGAAAATTCACCGAGACATAAAAGCTGCC
AATGTCTTGCTCTCAGAACAAGGAGATGTTAAACTTGCTGATTTTGGAGTTGCTGGTCAGCTGACAGATACACAG
10 ATTAAAGAAATACCTTTGIGGGAAGTCCATTTGGATGGCTCCTGAAGTTATTCAACAGTCAGCTTATGACTCA
AAAGCTGACATTTGGTCATTGGGAATTACTGCTATTGAACTAGCCAAGGGAGAGCCACCTAACTCCGATATGCAT
CCAATGAGAGTTCTGTTTCTTATCCCAAAAACAATCCTCCAAGTCTTGTGGAGACTTTACTAAGTCTTTTAAG
GAGTTTATTGATGCTTGCCTGAACAAAGATCCATCATTTTCGTCCTACAGCAAAGAAGTCTTGAAACACAAATTC
ATTGTAAAAAATTCAAAGAAGACTTCTTATCTGACTGAACTGATAGATCGTTTTAAGAGATGGAAGGCAGAAGGA
15 CACAGTGATGATGAATCTGATTCCGAGGGCTCTGATTTCGGAATCTACCAGCAGGGAAAACAATACTCATCCTGAA
TGGAGCTTTACCACCGTACGAAAGAAGCCTGATCCAAAGAAAGTACAGAATGGGGCAGAGCAAGATCTTGTGCAA
ACCCTGAGTTGTTTGTCTATGATAATCACACCTGCATTTGCTGAACTTAAACAGCAGGACGAGAATAACGCTAGC
AGGAATCAGGCGATTGAAGAAGTCTGAGAAAAGTATTGCTGTGGCTGAAGCCGCTGTCCCGGCATCACAGATAAA
ATGGTGAAGAACTAATTGAAAAATTTCAAAGTGTTTCAGCAGACGAATCCCCCTAAGAACTTATTATTGGCTT
20 CTGTTTCATATGGACCCAGAGAGCCCCACCAAACCTACGTCAAGATTAACAATGCTTAACCCATGAGCTCCATGT
GCC 3'

SEQ ID NO:8

25

10/18

FIG.9

5' GTCTATGATAATCACACCTGC 3'

5

SEQ ID NO:9

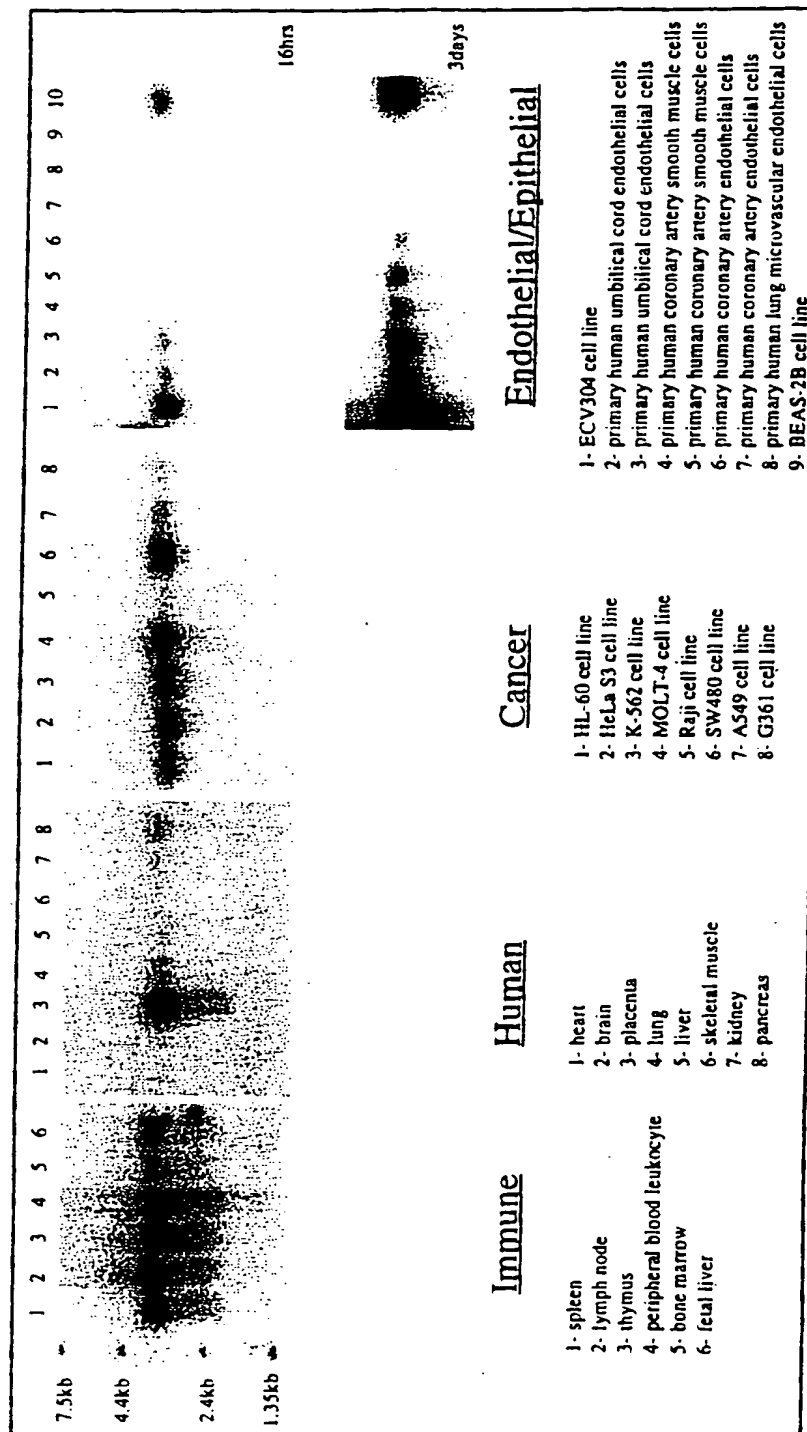
5' GGCACATGGAGCTCATGGGTTAAGC 3'

10

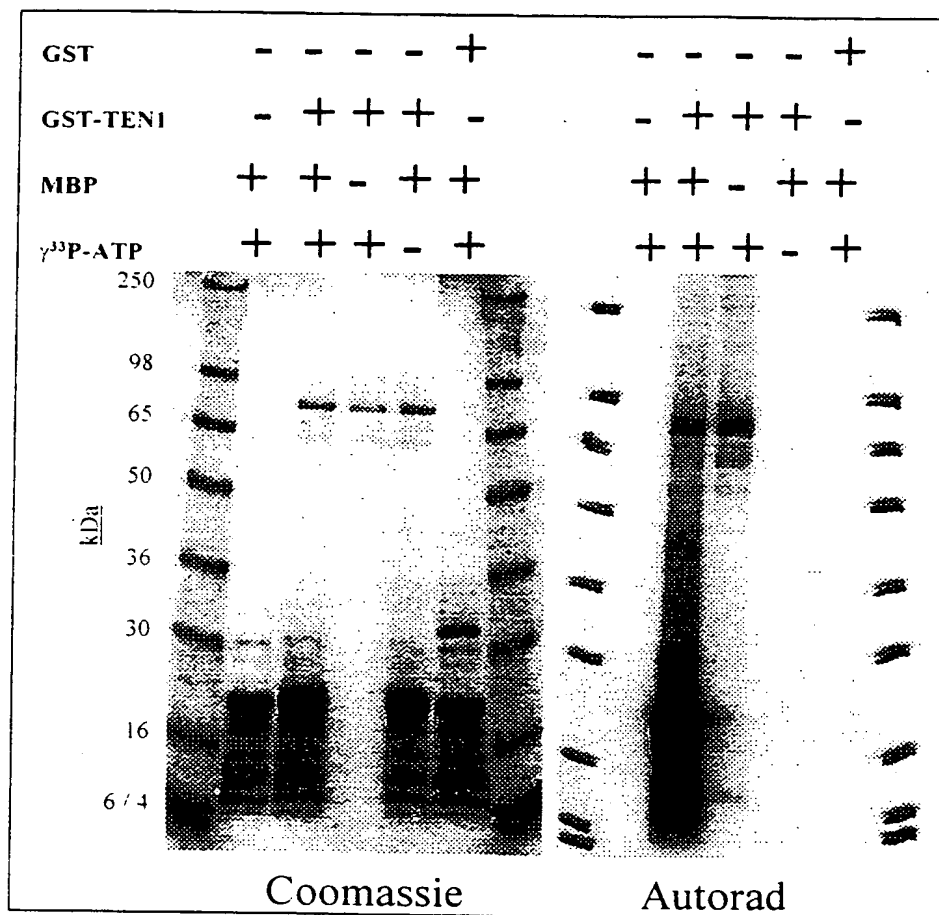
SEQ ID NO:10

11/18

FIG.10



12/18

FIG.11

13/18

FIG.12

MAHSPVAVQVPGMQNNIADPEELFTKLERICKGSFGEVFKGIDNRTQQVVAIKIIDLEEADEDEIEDIQQEITVLS
QCDSSYVTKYYSYLKGSKLWIIMEYLGGSALDLLRAGPFDEFQIATMLKEILKGLDYLHSEKKIHRDIKAANV
5 LLSEQGDVKLADFGVAGQLTDTQIKRNTFVGTPFWMAPEVIQQSAYDSKADIWSLGITAIELAKGEPPNSDMHPM
RVLFLIPKNNPPTLIGDFTKSFKFIDACLNKDPSFRPTAKELLKHKFIVKNSKKTSYLTEIDRFKRWKAEGHS
DEESDSEGSDSESSSRESNPHPEWSFTTVRKKPDPKKLQNGEEQDLVQTLSCLSMIITPAFAELKQODENNASRN
QAIEELEKSIABAETACPGITDKMVKKLIEKFQKCSADESP

10

SEQ ID NO:11

14/18

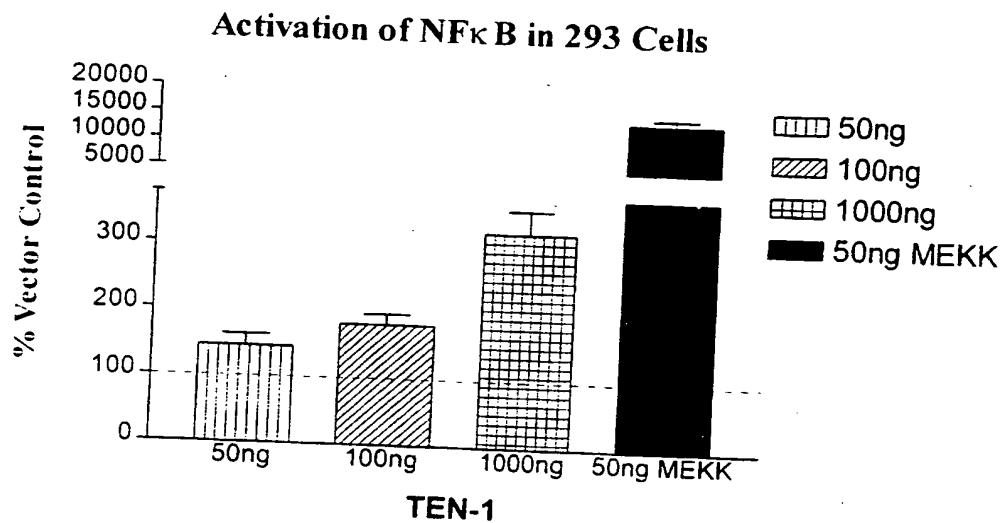
FIG.13

AGGCACCGCCACAGGTCAAGCCCTGCATT CAGGAAAGAGAGCAACACTGCAGTTAGCCAAAAGCCAGGCAGGCGAGCGGC
AGAGAGGCGCTCGATCGAGAAGCCTGGTAGAGCTGCAGAGATACCTCCGTAGGAGGAGCCAGTCTCTGCCGGAGGCGCCAC
5 CGCCACCACCGCAGAAGCAGCGCGAAGTAGCAGTCGCCACCATGGCCCACTCACCAGGTGGCTGTTCAAGTGCCTGGGATG
CAGAATAATATAGCAGATCCAGAAGAACTGTT CACAAAATTAGAGCGCATTGGAAAAGGCTCCTTTGGAGAAGTTTTCAA
AGGAATTGATAACCGTACTCAGCAAGTGGTTGCAATTAATAATCATTGACCTTGAGGAAGCTGAGGATGAAATAGAAGACA
TCCAACAAGAAATAACTGTTTTGAGTCAGTGCAGACAGCTCATATGTAACAAAATACTATGGGTCTATTTAAAGGGTTCA
AAACTATGGATAATAATGGAATACCTAGGTGGAGGTT CAGCATTGGATCTTCTGCGTGCTGGTCCATTTGATGAGTTCCA
10 GATTGCCACCATGCTCAAGGAGATTTTGAAAGGTCTGGACTATCTACATTCTGAAAAGAAAATCCACCGAGACATTAAAG
CTGCCAAGCTCTTGCTTTCAGAACAAGGTGATGTTAAACTGGCTGACTTTGGAGTTGCTGGCCAGCTGACAGATACACAA
ATCAAAAGAAACACCTTCGTAGGGACTCCGTTTTGGATGGCTCCTGAAGTTATTCAACAGTCAGCTTATGACTCTAAAGC
TGACATATGGTCTTTGGGAATTACTGCTATTGAACTTGCCAAGGGAGAGCCTCCGAATTCTGACATGCATCCAATGAGAG
TTCTGTTTCTTATTCAAAAAACAACCTCCAACCTCTTATTGGAGACTTTACTAAGTCTTTCAAGGAGTTTATTGATGCT
15 TGCCTGAATAAAGACCCGTCATTTCTGCTCTACAGCTAAAGAACTTTTGAAGCATAAGTTCATCGTAAAAAATTCAAAGAA
GACTTCTTATCTGACTGAATTGATCGATCGATTTAAGAGATGGAAGGCAGAAGGCCACAGTGATGAGGAATCTGATTCCG
AGGGCTCTGACTCGGAATCCAGCAGCAGGGAAAGTAACCCCTCACCCTGAATGGAGTTTCACCAGTGTGCGTAAGAAGCCT
GATCCAAAGAACTGCAGAATGGGGAAGAGCAAGATCTTGTGCAAACCTTGAGCTGTTTGTCTATGATAATCACACCTGC
ATTTGCCGAACCTTAAACAGCAGGACGAGAATAATGCGAGTCGAAACCAGGCAATTGAAGAACTTGAGAAAAGTATTGCTG
20 TGGCTGAAACCGCCTGTCTGGCATCACAGATAAGATGGTGAAGAACTAATCGAAAAATTTCAAAGTGTCTGCGGAT
GAATCCCCTTAAGAAATCTGTTGTCATTACTTTTGGCTTCTGTTTCATGTGGACCAGGAGAAACCCACCAAAGCTATGTC
AACCTTATAAATGCTTAACCTCATGAGCTCCATGTGCCTTTTGGATCTTTGCCACATTGAAGATTTAGAGGAAGCTATTAA
ACTATTTTGTGATGGTGATTATCATTTTGTATTTTAAAGAGATTATTTTGAAGGAATAATTTTAACTATAGTTTTTGC
CGGTATTGTAGTAAATGCTGAGATACAGGTTTTTTGTTTTTTGTTTTTTAATTTTAGGTACCATTATTTCTTATGTTTCAT
25 GGAATGAATACTGTTTGGTTTGGAACTCTTAGTTAACTGTATACTCATAAACATACAGGTCTTTCAAAGTCATCCTAACT
ATTAATGTTTGTAAATCATCAAGCTTCAAAAAGCATTCTTTTTCCCCACACAAGTATATTCTAAAAATGACTATTTGT
AATGAGGTGGAAGTAAGTAATACCTTCTTAAACAAGTGTTTTTAAGAAGCTCCCGGAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAA

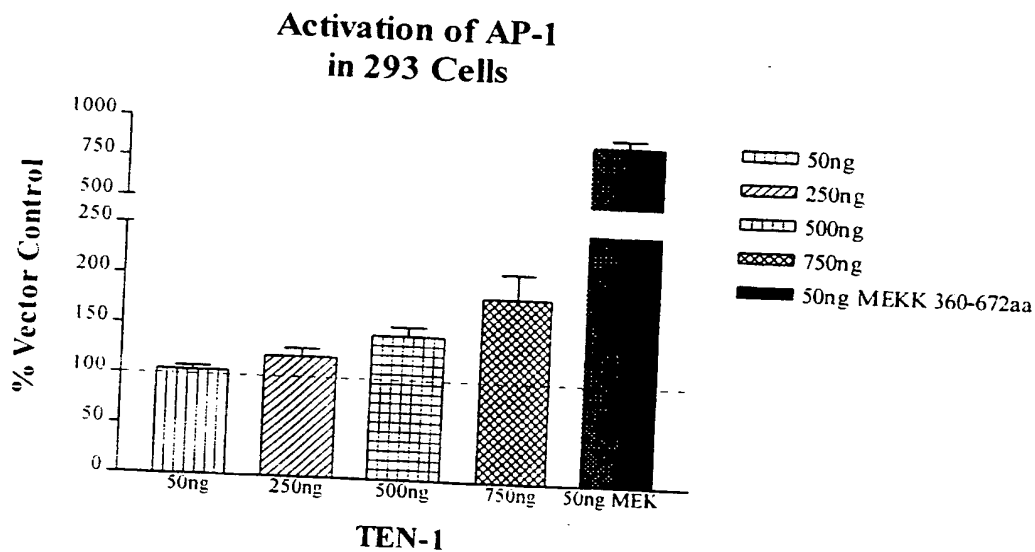
30

SEQ ID NO:12

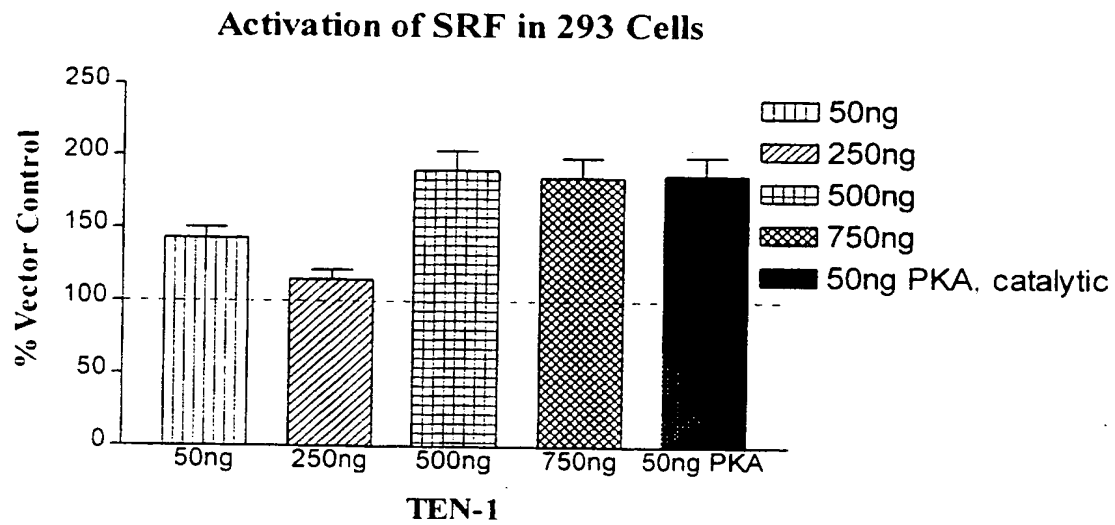
15/18

FIG.14

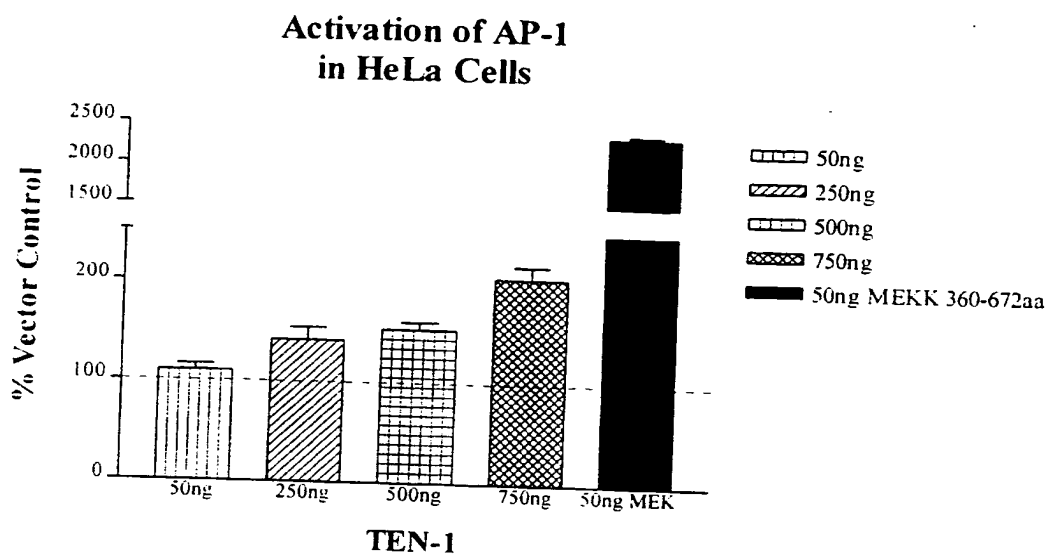
5



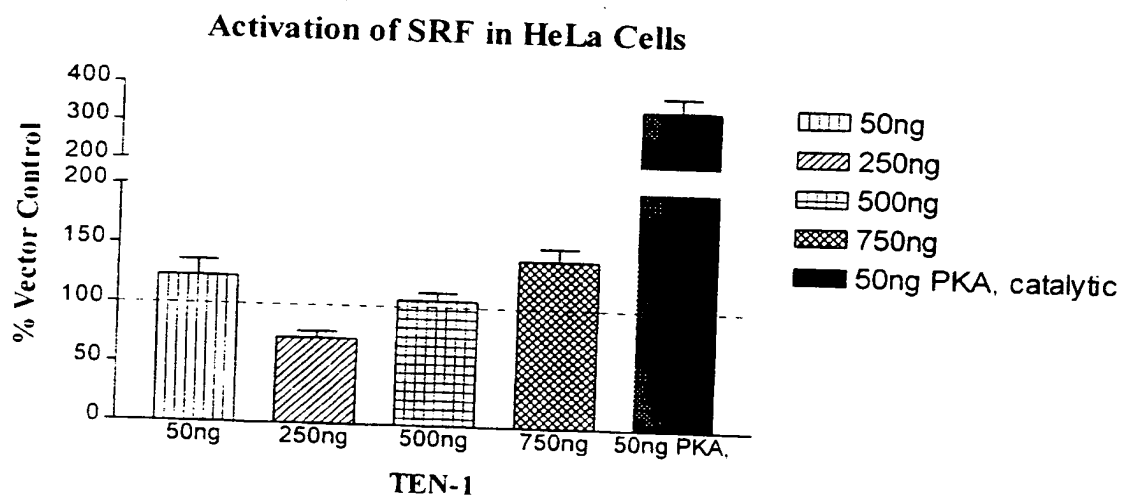
16/18

FIG.14 (continued)

17/18

FIG.15

5

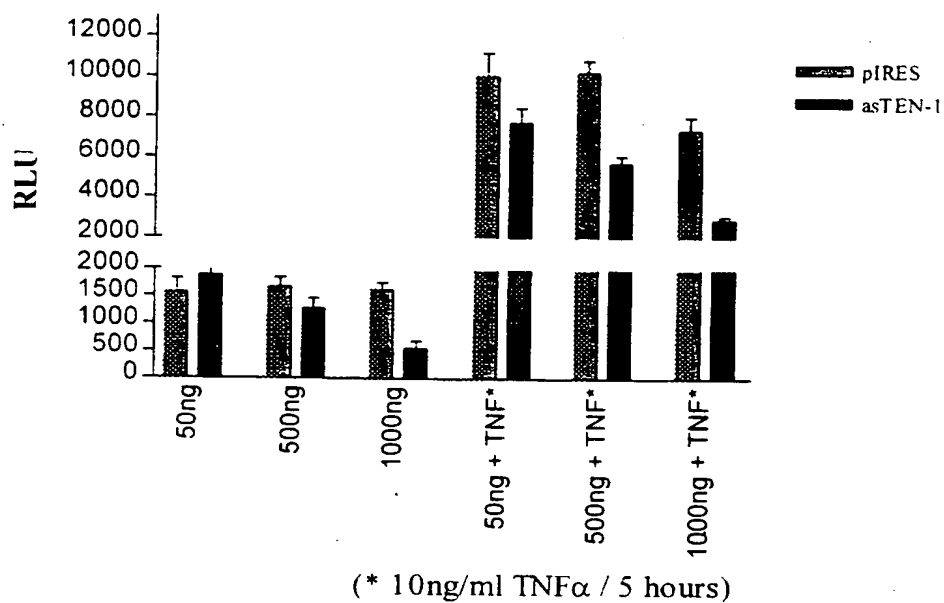


10

18/18

FIG.16

**Effect of Anisense TEN-1 cDNA
Expression on Basal Level and
TNF α -Mediated NF κ B Activation
in ECV304 Cells**



SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT:

- (A) NAME: Zeneca Ltd
- (B) STREET: 15 Stanhope Gate
- (C) CITY: London
- 10 (D) STATE: Greater London
- (E) COUNTRY: England
- (F) POSTAL CODE (ZIP): W1Y 6LN
- (G) TELEPHONE: 0171 304 5000
- 15 (H) TELEFAX: 0171 304 5151
- (I) TELEX: 0171 834 2042

(ii) TITLE OF INVENTION: PROCESS

20

(iii) NUMBER OF SEQUENCES: 12

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- 25 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: US 9726851.0

30

(vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: GB 9726851.0
- (B) FILING DATE: 19-DEC-1997

35

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3200 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

	TAACAGCCCCA CCTCCTAGCC CCGGGCTACG CGCCGCCAGC CCAGTAACCC CACTTTTGTG	60
5	TGTCCTCCCA GGCCCCGATC GAAAAGCCTG GGAGGGCCGC CGAACTACCC CCGGAGGGAG	120
	GAGCCAGTCC GAACCCAAGG CGCCACCGCC GCAGAAGCGG AGCGAGGCAG CATTGCGCTC	180
10	CATGGCCCAC TCGCCGGTGG CTGTCCAAGT GCCTGGGATG CAGAATAACA TAGCTGATCC	240
	AGAAGAACTG TTCACAAAAT TAGAGCGCAT TGGGAAAGGC TCATTGGGGG AAGTTTTCAA	300
	AGGAATTGAT AACCGTACCC AGCAAGTCGT TGCTATTAAA ATCATAGACC TTGAGGAAGC	360
15	CGAAGATGAA ATAGAAGACA TTCAGCAAGA AATAACTGTC TTGAGTCAAT GTGACAGCTC	420
	ATATGTAACA AAATACTATG GGTCAATTTT AAAGGGGTCT AAATTATGGA TAATAATGGA	480
20	ATACCTGGGC GGTGGTTCAG CACTGGATCT TCTTCGAGCT GGTCCATTG ATGAGTTCCA	540
	GATTGCTACC ATGCTAAAGG AAATTTTAAA AGGTCTGGAC TATCTGCATT CAGAAAAGAA	600
	AATTCACCGA GACATAAAG CTGCCAATGT CTTGCTCTCA GAACAAGGAG ATGTTAAACT	660
25	TGCTGATTTT GGAGTTGCTG GTCAGCTGAC AGATACACAG ATTAAAAGAA ATACCTTTGT	720
	GGGAACCCA TTTTGGATGG CTCTGAAGT TATTCAACAG TCAGCTTATG ACTCAAAGC	780
30	TGACATTTGG TCATTGGGAA TTAGTCTAT TGAAGTAGCC AAGGGAGAGC CACCTAACTC	840
	CGATATGCAT CCAATGAGAG TTCTGTTTCT TATTCCCAA AACAACTCTC CAACTCTTGT	900
	TGGAGACTTT ACTAAGTCTT TTAAGGAGTT TATTGATGCT TGCCTGAACA AAGATCCATC	960
35	ATTTGCTCCT ACAGCAAAAG AACTTCTGAA ACACAAATTC ATTGTAAAAA ATTCAAAGAA	1020
	GACTTCTTAT CTGACTGAAC TGATAGATCG TTTAAGAGA TGGAAGGCAG AAGGACACAG	1080
40	TGATGATGAA TCTGATTCCG AGGGCTCTGA TTCGGAATCT ACCAGCAGGG AAAACAATA	1140
	TCATCCTGAA TGGAGCTTTA CCACCGTACG AAAGAAGCCT GATCCAAAGA AAGTACAGAA	1200
	TGGGGCAGAG CAAGATCTTG TGCAAACCTG AGTTGTTTGT CTATGATAAT CACACCTGCA	1260
45	TTTGCTGAAC TTAAACAGCA GGACGAGAAT AACGCTAGCA GGAATCAGGC GATTGAAGAA	1320

	CTCGAGAAAA GTATTGCTGT GGCTGAAGCC GCCTGTCCCG GCATCACAGA TAAATGGTG	1380
	AAGAACTAA TTGAAAAATT TCAAAAGTGT TCAGCAGACG AATCCCCCTA AGAACTTAT	1440
5	TATTGGCTTC TGTTCATAT GGACCCAGAG AGCCCCACCA AACCTACGTC AAGATTAAAC	1500
	ATGCTTAACC CATGAGCTCC ATGTGCCTTT TGGATCTTTG CAACACTGAA GATTTGGAAG	1560
10	AAGCTATTAA ACTATTTTGT GATGGCGTTT ATCATTTTAT ATTTTGAAAG GATTATTTTG	1620
	TAAGGAATAA CTTTAAATAC TATAGTTTCA CCTGTATTCT AGTAAATGTT GAGACACCGT	1680
	TTTGCTTTTA AGTATCCCTA TTTCTTAAGT TACGAGGATG AATACCTTTC ACATTTTGAT	1740
15	CTTTAGTTGA CTCTACAGTC ATGAAACATA CAGGTCTTTC AAAGTCATTC TCAATATTCA	1800
	GCTTTTGTA ATTATCAAGC TTCAAAAAGC TTTTTTTTAA AAAAAAAAC ATGCATATTC	1860
20	TAAAAATGAC TATTGGGTGG GGAGGTGTAA ATAAGTCATA CCTTCTTAA ACAGAAAATT	1920
	TAAGTAAAGT CTTTAAATG AAACCTGTAA AAGTATTGAC TCTTCTACCA AGTTGGTATG	1980
	ATATTCCAGG CAGCTCAATG ATTATCACAT TTGAGACCCT GTGTTTGAAG CATTTACAGG	2040
25	CAATGTACAG CAACAGAGGT ACCTCTTGGT GTATAGTATT TACATTCTCT TTTAGGTAGA	2100
	AGAGGCAATT TTACCCTTAT TTCACATGGT TAGAAATTTA AAGCAAGATC ATTTACCCAA	2160
30	GGATAGGTGT TTGGAATGT TGAAGGAGTT AGTCTGGCTT CATGTTTTAC ATCTTCAACT	2220
	AAAATCCCAT ACTATCTGCT TGGATTGGA GAGCCAAAAA ATAAAGCTGA TTGTCATGTG	2280
	ATTAAATATC TGATCAACAG GTATGAATAT AACTTAAATC AGCATATTTT TGCCATGGTA	2340
35	ATAAATGTC CTATAAACTA TTTATATATT TTTGTTCTTC ATAATTATCA CTAATAAGCA	2400
	TCAGTTTGT GTTTTTAAAA GGATAATTTA GTGAGCATTT TCTAGTTCAT ATGAAAATAA	2460
40	CCATAGTACA GGATGATTC TGTCCACACA AAGGTTAAAT TAGATTGCAC AGTTAATTTT	2520
	CACTTATATT TATGGTACTA TTATGIGGGT GATGCCTTTT TCTTTAAGC CCAGTACATA	2580
	TATTATGCT GCCTAAGTTC TGAAGTGGG CTGTATTTC AAGTTGTAG AATTATTGAT	2640
45	ATTTAGTTTT GATAGCTAAT GTTTAATTGT TTGGATCTGC ACAGTTGGT TTTTGCACAA	2700

-4-

AAGTCATTTA AAAAAATCTG AGTAATTGTC AAATATTAAA AGAAAGATAT TCTTCCTGTA 2760
AGGAATACAG TTTTGTAGCA AAGTGGCCAT TACATCCTCT TTTTAATTTA CATAATACAG 2820
5 ATACTTGAGA AAGTTGTTGT GGTGTTGTAT GCCAAGAAAA TTCTTTTTAT TGGTGCCTAT 2880
ATTGTAACAA TTATTTTAA TGCATTGTAT TTTGAAGTAA CGGTTTCAGTT AAATTTTCA 2940
CCTGCTGTGT AACTGAAACA CAATTACAGT TTATAATCAT CTGTAGAAGT CTGGAGATAA 3000
10 TTTTGCAACT CATGTTATGG GTTAAATGAA TATTTTGTGA AAAGTAAAAG CAACAAATTT 3060
ATAAATTGAT TATTTGAAAC TTTACAACAC AATTGCATCC CAAATACAAA TTGTATTGCT 3120
15 TATTCATTAT AGCTATTCGT CCTGTAATCT GTTCTAGGT GAAGCATACT CCAGTGTGTTT 3180
AGGGGTTTTG AAAATAAATA 3200

(2) INFORMATION FOR SEQ ID NO: 2:

20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1251 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

25

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATGGCCCACT CGCCGGTGGC TGTCCAAGTG CCTGGGATGC AGAATAACAT AGCTGATCCA 60
35 GAAGAACTGT TCACAAAATT AGAGCGCATT GGGAAAGGCT CATTTGGGGA AGTTTTCAAA 120
GGAATTGATA ACCGTACCCA GCAAGTCGTT GCTATTAAAA TCATAGACCT TGAGGAAGCC 180
40 GAAGATGAAA TAGAAGACAT TCAGCAAGAA ATAAGTGTCT TGAGTCAATG TGACAGCTCA 240
TATGTAACAA AATACTATGG GTCATATTTA AAGGGGTCTA AATTATGGAT AATAATGGAA 300
TACCTGGGCG GTGGTTCAGC ACTGATCTT CTCGAGCTG GTCCATTGTA TGAGTTCCAG 360
45 ATTGCTACCA TGCTAAAGGA AATTTTAAAA GGTCTGGAAT ATCTGCAATC AGAAAAGAAA 420

-5-

ATTACCGAG ACATAAAAGC TGCCAATGTC TTGCTCTCAG AACAAGGAGA TGTAAACTT 480
GCTGATTTTG GAGTTGCTGG TCAGCTGACA GATACACAGA TTAAGAGAAA TACCTTTGTG 540
5 GGAAGTCCAT TTGGGATGGC TCCTGAAGTT ATTCAACAGT CAGCTTATGA CTCAAAGCT 600
GACATTTGGT CATTGGGAAT TACTGCTATT GAACTAGCCA AGGGAGAGCC ACCTAACTCC 660
GATATGCATC CAATGAGAGT TCTGTTTCTT ATTCCCAAAA ACAATCCTCC AACTCTTGTT 720
10 GGAGACTTAA CTAAGTCTTT TAAGGAGTTT ATTGATGCTT GCCTGAACAA AGATCCATCA 780
TTTCGTCTTA CAGCAAAAGA ACTTCTGAAA CACAAATTCA TTGTAAAAAA TTCAAAGAAG 840
15 ACTTCTTATC TGAAGTGAAT GATAGATCGT TTTAAGAGAT GGAAGGCAGA AGGACACAGT 900
GATGATGAAT CTGATTCCGA GGGCTCTGAT TCGGAATCTA CCAGCAGGGA AAACAATACT 960
CATCTGAAAT GGAGCTTTAC CACCGTACGA AAGAAGCCTG ATCCAAAGAA AGTACAGAAT 1020
20 GGGGCAGAGC AAGATCTTGT GCAAACCCTG AGTTGTTTGT CTATGATAAT CACACCTGCA 1080
TTTGCTGAAC TTAAACAGCA GGACGAGAAT AACGCTAGCA GGAATCAGGC GATTGAAGAA 1140
25 CTCGAGAAAA GTATTGCTGT GGCTGAAGCC GCCTGTCCCG GCATCACAGA TAAATGGTG 1200
AAGAACTAA TTGAAAAATT TCAAAAGTGT TCAGCAGAGC AATCCCCCTA A 1251

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 416 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Ala His Ser Pro Val Ala Val Gln Val Pro Gly Met Gln Asn Asn
1 5 10 15

-6-

Ile Ala Asp Pro Glu Glu Leu Phe Thr Lys Leu Glu Arg Ile Gly Lys
 20 25 30

5 Gly Ser Phe Gly Glu Val Phe Lys Gly Ile Asp Asn Arg Thr Gln Gln
 35 40 45

Val Val Ala Ile Lys Ile Ile Asp Leu Glu Glu Ala Glu Asp Glu Ile
 50 55 60

10 Glu Asp Ile Gln Gln Glu Ile Thr Val Leu Ser Gln Cys Asp Ser Ser
 65 70 75 80

Tyr Val Thr Lys Tyr Tyr Gly Ser Tyr Leu Lys Gly Ser Lys Leu Trp
 85 90 95

15 Ile Ile Met Glu Tyr Leu Gly Gly Gly Ser Ala Leu Asp Leu Leu Arg
 100 105 110

Ala Gly Pro Phe Asp Glu Phe Gln Ile Ala Thr Met Leu Lys Glu Ile
 115 120 125

Leu Lys Gly Leu Asp Tyr Leu His Ser Glu Lys Lys Ile His Arg Asp
 130 135 140

25 Ile Lys Ala Ala Asn Val Leu Leu Ser Glu Gln Gly Asp Val Lys Leu
 145 150 155 160

Ala Asp Phe Gly Val Ala Gly Gln Leu Thr Asp Thr Gln Ile Lys Arg
 165 170 175

30 Asn Thr Phe Val Gly Thr Pro Phe Trp Met Ala Pro Glu Val Ile Gln
 180 185 190

Gln Ser Ala Tyr Asp Ser Lys Ala Asp Ile Trp Ser Leu Gly Ile Thr
 195 200 205

Ala Ile Glu Leu Ala Lys Gly Glu Pro Pro Asn Ser Asp Met His Pro
 210 215 220

40 Met Arg Val Leu Phe Leu Ile Pro Lys Asn Asn Pro Pro Thr Leu Val
 225 230 235 240

Gly Asp Phe Thr Lys Ser Phe Lys Glu Phe Ile Asp Ala Cys Leu Asn
 245 250 255

45 Lys Asp Pro Ser Phe Arg Pro Thr Ala Lys Glu Leu Leu Lys His Lys

-7-

	260	265	270
5	Phe Ile Val Lys Asn Ser Lys Lys Thr Ser Tyr Leu Thr Glu Leu Ile 275 280 285		
	Asp Arg Phe Lys Arg Trp Lys Ala Glu Gly His Ser Asp Asp Glu Ser 290 295 300		
10	Asp Ser Glu Gly Ser Asp Ser Glu Ser Thr Ser Arg Glu Asn Asn Thr 305 310 315 320		
	His Pro Glu Trp Ser Phe Thr Thr Val Arg Lys Lys Pro Asp Pro Lys 325 330 335		
15	Lys Val Gln Asn Gly Ala Glu Gln Asp Leu Val Gln Thr Leu Ser Cys 340 345 350		
20	Leu Ser Met Ile Ile Thr Pro Ala Phe Ala Glu Leu Lys Gln Gln Asp 355 360 365		
	Glu Asn Asn Ala Ser Arg Asn Gln Ala Ile Glu Glu Leu Glu Lys Ser 370 375 380		
25	Ile Ala Val Ala Glu Ala Ala Cys Pro Gly Ile Thr Asp Lys Met Val 385 390 395 400		
	Lys Lys Leu Ile Glu Lys Phe Gln Lys Cys Ser Ala Asp Glu Ser Pro 405 410 415		

(2) INFORMATION FOR SEQ ID NO: 4:

(2) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 426 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Ala His Leu Arg Gly Phe Ala Asn Gln His Ser Arg Val Asp Pro

-8-

1 5 10 15
 Glu Glu Leu Phe Thr Lys Leu Asp Arg Ile Gly Lys Gly Ser Phe Gly
 20 25 30
 5
 Glu Val Tyr Lys Gly Ile Asp Asn His Thr Lys Glu Val Val Ala Ile
 35 40 45
 Lys Ile Ile Asp Leu Glu Glu Ala Glu Asp Glu Ile Glu Asp Ile Gln
 10 50 55 60
 Gln Glu Ile Thr Val Leu Ser Gln Cys Asp Ser Pro Tyr Ile Thr Arg
 65 70 75 80
 15
 Tyr Phe Gly Ser Tyr Leu Lys Ser Thr Lys Leu Trp Ile Ile Met Glu
 85 90 95
 Tyr Leu Gly Gly Gly Ser Ala Leu Asp Leu Leu Lys Pro Gly Pro Leu
 100 105 110
 20
 Glu Glu Thr Tyr Ile Ala Thr Ile Leu Arg Glu Ile Leu Lys Gly Leu
 115 120 125
 Asp Tyr Leu His Ser Glu Arg Lys Ile His Arg Asp Ile Lys Ala Ala
 25 130 135 140
 Asn Val Leu Leu Ser Glu Gln Gly Asp Val Lys Leu Ala Asp Phe Gly
 145 150 155 160
 30
 Val Ala Gly Gln Leu Thr Asp Thr Gln Ile Lys Arg Asn Thr Phe Val
 165 170 175
 Gly Thr Pro Phe Trp Met Ala Pro Glu Val Ile Lys Gln Ser Ala Tyr
 180 185 190
 35
 Asp Phe Lys Ala Asp Ile Trp Ser Leu Gly Ile Thr Ala Ile Glu Leu
 195 200 205
 Ala Lys Gly Glu Pro Pro Asn Ser Asp Leu His Pro Met Arg Val Leu
 40 210 215 220
 Phe Leu Ile Pro Lys Asn Ser Pro Pro Thr Leu Glu Gly Gln His Ser
 225 230 235 240
 45
 Lys Pro Phe Lys Glu Phe Val Glu Ala Cys Leu Asn Lys Asp Pro Arg
 245 250 255

-9-

Phe Arg Pro Thr Ala Lys Glu Leu Leu Lys His Lys Phe Ile Thr Arg
 260 265 270

5 Tyr Thr Lys Lys Thr Ser Phe Leu Thr Glu Leu Ile Asp Arg Tyr Lys
 275 280 285

Arg Trp Lys Ser Glu Gly His Gly Glu Glu Ser Ser Ser Glu Asp Ser
 290 295 300

10 Asp Ile Asp Gly Glu Ala Glu Asp Gly Glu Gln Gly Pro Ile Trp Thr
 305 310 315 320

Phe Pro Pro Thr Ile Arg Pro Ser Pro His Ser Lys Leu His Lys Gly
 325 330 335

15 Thr Ala Leu His Ser Ser Gln Lys Pro Ala Asp Ala Val Lys Arg Gln
 340 345 350

Pro Arg Ser Gln Cys Leu Ser Thr Leu Val Arg Pro Val Phe Gly Glu
 20 355 360 365

Leu Lys Glu Lys His Lys Gln Ser Gly Gly Ser Val Gly Ala Leu Glu
 370 375 380

25 Glu Leu Glu Asn Ala Phe Ser Leu Ala Glu Glu Ser Cys Pro Gly Ile
 385 390 395 400

Ser Asp Lys Leu Met Val His Leu Val Glu Arg Val Gln Arg Phe Ser
 30 405 410 415

His Asn Arg Asn His Leu Thr Ser Thr Arg
 420 425

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 431 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

-10-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

5	Met Ala His Ser Pro Val Gln Ser Gly Leu Pro Gly Met Gln Asn Leu	1	5	10	15
	Lys Ala Asp Pro Glu Glu Leu Phe Thr Lys Leu Glu Lys Ile Gly Lys	20	25	30	
10	Gly Ser Phe Gly Glu Val Phe Lys Gly Ile Asp Asn Arg Thr Gln Lys	35	40	45	
	Val Val Ala Ile Lys Ile Ile Asp Leu Glu Glu Ala Glu Asp Glu Ile	50	55	60	
15	Glu Asp Ile Gln Gln Glu Ile Thr Val Leu Ser Gln Cys Asp Ser Pro	65	70	75	80
	Tyr Val Thr Lys Tyr Tyr Gly Ser Tyr Leu Lys Asp Thr Lys Leu Trp	85	90	95	
20	Ile Ile Met Glu Tyr Leu Gly Gly Gly Ser Ala Leu Asp Leu Leu Glu	100	105	110	
	Pro Gly Pro Leu Asp Glu Thr Gln Ile Ala Thr Ile Leu Arg Glu Ile	115	120	125	
25	Leu Lys Gly Leu Asp Tyr Leu His Ser Glu Lys Lys Ile His Arg Asp	130	135	140	
30	Ile Lys Ala Ala Asn Val Leu Leu Ser Glu His Gly Glu Val Lys Leu	145	150	155	160
	Ala Asp Phe Gly Val Ala Gly Gln Leu Thr Asp Thr Gln Ile Lys Arg	165	170	175	
35	Asn Thr Phe Val Gly Thr Pro Phe Trp Met Ala Pro Glu Val Ile Lys	180	185	190	
	Gln Ser Ala Tyr Asp Ser Lys Ala Asp Ile Trp Ser Leu Gly Ile Thr	195	200	205	
40	Ala Ile Glu Leu Ala Arg Gly Glu Pro Pro His Ser Glu Leu His Pro	210	215	220	
45	Met Lys Val Leu Phe Leu Ile Pro Lys Asn Asn Pro Pro Thr Leu Glu	225	230	235	240

-11-

Gly Asn Tyr Ser Lys Pro Leu Lys Glu Phe Val Glu Ala Cys Leu Asn
 245 250 255

5 Lys Glu Pro Ser Phe Arg Pro Thr Ala Lys Glu Leu Leu Lys His Lys
 260 265 270

Phe Ile Leu Arg Asn Ala Lys Lys Thr Ser Tyr Leu Thr Glu Leu Ile
 275 280 285

10 Asp Arg Tyr Lys Arg Trp Lys Ala Glu Gln Ser His Asp Asp Ser Ser
 290 295 300

Ser Glu Asp Ser Asp Ala Glu Thr Asp Gly Gln Ala Ser Gly Gly Ser
 305 310 315 320

15 Asp Ser Gly Asp Trp Ile Phe Thr Ile Arg Glu Lys Asp Pro Lys Asn
 325 330 335

Leu Glu Asn Gly Ala Leu Gln Pro Ser Asp Leu Asp Arg Asn Lys Met
 340 345 350

Lys Asp Ile Pro Lys Arg Pro Phe Ser Gln Cys Leu Ser Thr Ile Ile
 355 360 365

25 Ser Pro Leu Phe Ala Glu Leu Lys Glu Lys Ser Gln Ala Cys Gly Gly
 370 375 380

Asn Leu Gly Ser Ile Glu Glu Leu Arg Gly Ala Ile Tyr Leu Ala Glu
 385 390 395 400

30 Glu Val Cys Pro Gly Ile Ser Asp Thr Met Val Ala Gln Leu Val Gln
 405 410 415

Arg Leu Gln Arg Tyr Ser Leu Ser Gly Gly Gly Thr Ser Ser His
 420 425 430

35

(2) INFORMATION FOR SEQ ID NO: 6:

- 40 (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 45 (ii) MOLECULE TYPE: other nucleic acid

-12-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GCCTCCATGG CCCACTCGCC GGTG

24

5 (2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

20 GGCACATGGA GTCATGGGT TAAGC

25

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 1353 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: other nucleic acid

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GCCTCCATGG CCCACTCGCC GGTGGCTGTC CAAGTGCCTG GGATGCAGAA TAACATAGCT 60

40 GATCCAGAAG AACTGTTTAC AAAATTAGAG CGCATTGGGA AAGGCTCATT TGGGGAAGTT 120

TTCAAAGGAA TTGATAACCG TACCCAGCAA GTCGTTGCTA TTAAAATCAT AGACCTTGAG 180

GAAGCCGAAG ATGAAATAGA AGACATTCAG CAAGAAATAA CTGTCTTGAG TCAATGTGAC 240

45 AGCTCATATG TAACAAAATA CTATGGGTCA TATTAAAGG GGTCTAAATT ATGGATAATA 300

ATGGAATACC TGGGCGGTGG TTCAGCACTG GATCTTCTTC GAGCTGGTCC ATTTGATGAG 360
 TTCCAGATTG CTACCATGCT AAAGGAAATT TTAAAAGGTC TGGACTATCT GCATTCAGAA 420
 5 AAGAAAATTC ACCGAGACAT AAAAGCTGCC AATGTCTTGC TCTCAGAACA AGGAGATGTT 480
 AAACCTGCTG ATTTTGGAGT TGCTGGTCAG CTGACAGATA CACAGATTAA AAGAAAATACC 540
 TTTGTGGGAA CTCCATTTTG GATGGCTCCT GAAGTTATTC AACAGTCAGC TTATGACTCA 600
 10 AAAGCTGACA TTTGGTCATT GGAATTACT GCTATTGAAC TAGCCAAGGG AGAGCCACCT 660
 AACTCCGATA TGCATCCAAT GAGAGTTCTG TTTCTTATTC CCAAAAACAA TCCTCCAAC 720
 15 CTGTGTGAG ACTTTACTAA GTCTTTTAA GAGTTTATTG ATGCTTGCTT GAACAAAGAT 780
 CCATCATTTC GTCTACAGC AAAAGAACTT CTGAAACACA AATTCATTGT AAAAAATTCA 840
 AAGAAGACTT CTTAICTGAC TGAAGTATA GATCGTTTTA AGAGATGGAA GGCAGAAGGA 900
 20 CACAGTGATG ATGAATCTGA TTCCGAGGGC TCTGATTGGG AATCTACCAG CAGGGAAAAC 960
 AATACTCATC CTGAATGGAG CTTTACCACC GTACGAAAGA AGCCTGATCC AAAGAAAGTA 1020
 25 CAGAATGGGG CAGAGCAAGA TCTTGTGCAA ACCCTGAGTT GTTTGTCTAT GATAATCACA 1080
 CCTGCATTTC CTGAACTTAA ACAGCAGGAC GAGAATAACG CTAGCAGGAA TCAGGCGATT 1140
 GAAGAACTCG AGAAAAGTAT TGCTGTGGCT GAAGCCGCCT GTCCCGGCAT CACAGATAAA 1200
 30 ATGGTGAAGA AACTAATTGA AAAATTTCAA AAGTGTTTCA CAGACGAATC CCCCTAAGAA 1260
 ACTTATTATT GGCTTCTGTT TCATATGGAC CCAGAGAGCC CCACCAAACC TACGTCAAGA 1320
 35 TTAACAATGC TTAACCCATG AGCTCCATGT GCC 1353

(2) INFORMATION FOR SEQ ID NO: 9:

- 40 (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 45 (ii) MOLECULE TYPE: other nucleic acid

-14-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GTCTATGATA ATCACACCTG C

21

5 (2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

20 GGCACATGGA GCTCATGGGT TAAGC

25

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 77 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: other nucleic acid

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

AAGATGGGGT AATCTGGGGG AAGATGAAGA GAGTTTGTAA AGTAAGTGTA CTATTAGGTT

60

GTCTAAAAAA TAGGTCA

77

40

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2028 base pairs

45 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

-15-

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

10	AGGCACCGCC ACAGGTCAAG CCCTGCATTC AGGAAAGAGA GCAACACTGC AGTTAGCCAA	60
	AAGCCAGGCA GGCGAGCGGC AGAGAGGCCT CGATCGAGAA GCCTGGTAGA GCTGCAGAGA	120
	TACCTCCGTA GGAGGAGCCA GTCTCTGCCG GAGGCGCCAC CGCCACCACC GCAGAAGCAG	180
15	CGCGAAGTAG CAGTCGCCAC CATGGCCCAC TCACCGGTGG CTGTTCAAGT GCCTGGGATG	240
	CAGAATAATA TAGCAGATCC AGAAGAACTG TTCACAAAAT TAGAGCGCAT TGGAAAAGGC	300
20	TCCTTTGGAG AAGTTTTCAA AGGAATTGAT AACCGTACTC AGCAAGTGGT TGCAATTAAA	360
	ATCATTGACC TTGAGGAAGC TGAGGATGAA ATAGAAGACA TCCAACAAGA AATAACTGTT	420
	TTGAGTCAGT GCGACAGCTC ATATGTAACA AAATACTATG GGTCCATATT AAAGGGTTCA	480
25	AAACTATGGA TAATAATGGA ATACCTAGGT GGAGSTTCAG CATTGGATCT TCTGCGTGCT	540
	GGTCCATTTC ATGAGTTCCA GATTGCCACC ATGCTCAAGG AGATTTTGAA AGGTCTGGAC	600
30	TATCTACATT CTGAAAAGAA AATCCACCGA GACATTAAAG CTGCCAACGT CTTGCTTTCA	660
	GAACAAGGTG ATGTTAAACT GGCTGACTTT GGAGTTGCTG GCCAGCTGAC AGATACACAA	720
	ATCAAAAGAA ACACCTTCGT AGGGACTCCG TTTTGGATGG CTCCTGAAGT TATTCAACAG	780
35	TCAGCTTATG ACTCTAAAGC TGACATATGG TCTTTGGGAA TTAGTGCTAT TGAAGTTGCC	840
	AAGGGAGAGC CTCCGAATTC TGACATGCAT CCAATGAGAG TTCTGTTTCT TATTCACAAA	900
40	AACAACCCCTC CAACTCTTAT TGGAGACTTT ACTAAGTCTT TCAAGGAGTT TATTGATGCT	960
	TGCCTGAATA AAGACCCGTC ATTTCGTCTT ACAGCTAAAG AACTTTTGAA GCATAAGTTC	1020
	ATCGTAAAAA ATTCAAAGAA GACTTCTTAT CTGACTGAAT TGATCGATCG ATTTAAGAGA	1080
45	TGGAAGGCAG AAGGCCACAG TGATGAGGAA TGTGATTCCG AGGGCTCTGA CTCGGAATCC	1140

-16-

AGCAGCAGGG AAAGTAACCC TCACCCTGAA TGGAGTTTCA CCACTGTGCG TAAGAAGCCT 1200

GATCCAAAGA AACTGCAGAA TGGGAAGAG CAAGATCTTG TGCAAACCTT GAGCTGTTTG 1260

5 TCTATGATAA TCACACCTGC ATTTGCCGAA CTAAACAGC AGGACGAGAA TAATGCGAGT 1320

CGAAACCAGG CAATTGAAGA ACTTGAGAAA AGTATTGCTG TGGCTGAAAC CGCCTGTCCT 1380

GGCATCACAG ATAAGATGGT GAAGAACTA ATCGAAAAAT TTCAAAGTG TTCTGCGGAT 1440

10 GAATCCCCTT AAGAAATCTG TTGTCATTAC TTTTGGCTTC TGTTTCATGT GGACCAGGAG 1500

AAACCCACCA AAGCTATGTC AACCTTATAA ATGCTTAACT CATGAGCTCC ATGTGCCTTT 1560

15 TGGATCTTTG CCACATTGAA GATTAGAGG AAGCTATTAA ACTATTTTGT GATGGTGATT 1620

ATCATTTTGT ATTTTAAAGA GATTATTTTG TAAGGAATAA TTTTAATACT ATAGTTTTC 1680

CGGTATTGTA GTAAATGCTG AGATACAGGT TTTTGTTTTT TTGTTTTTTA ATTTTAGGTA 1740

20 CCATTATTTT TATGTTTCAT GGAATGAATA CTGTTTGTTT TGAATCTTT AGTTAACTGT 1800

ATACTCATAA ACATACAGGT CTTTCAAAGT CATCCTAACT ATTAAATGTT TGTAATCAT 1860

25 CAAGCTTCAA AAAGCATTCT TTTTCCCCCA CACAAGTATA TTCTAAAAAT GACTATTTGT 1920

AATGAGGTGG AAGTAAGTAA TACCTTCTTA AAACAAGTGT TTTTAAGAAG CTCCCGGAAA 1980

AAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAA 2028

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 98/03793

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/54 C12N9/12 C12N15/11 C12N5/10 C07K16/40
G01N33/573 C12Q1/48 C12Q1/68 A61K31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K G01N C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCHINKMANN K. AND BLENIS J.: "Cloning and characterization of a human STE20-like protein kinase with unusual co-factor requirements." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 45, 7 November 1997, pages 28695-28703, XP002086764 cited in the application see the whole document --- -/--	1-11, 17-19



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

31 March 1999

Date of mailing of the international search report

16/04/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Mandl, B

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 98/03793

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL database entry HSZZ18948; accession number AA313812; 8. April 1997; Adams et al.: 'Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence.' XP002098714 see abstract ----	1-6,8,9, 19
X	EMBL database entry HS504336; accession number W16504; 4. May 1996; Hillier et al.: 'The WashU-Merck EST project.' XP002098715 see abstract ----	1-6,8,9, 19
X	EMBL database entry HSZZ14761; accession number AA309584; 18. April 1997; Adams et al.: 'Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence.' XP002098716 see abstract -----	4,19

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 98/03793

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 15 and 16 and claim 17 as far as an in vivo application is concerned are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 12-16
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 12-16

Said claims relate to compounds that modulate the activity of a signal-transduction kinase' and their use as medicaments without giving a detailed characterization of the claimed matter. Consequently, the scope of said claims is ambiguous and, moreover, their subject-matter is vague and not sufficiently disclosed.

THIS PAGE BLANK (USPTO)

THIS PAGE BLANK (USPTO)